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## Is Silent Ischemic Heart Disease Evident in Rheumatoid Arthritis Patients?

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**Abstract: Background:** Large number of studies have shown that individuals with rheumatoid arthritis (RA) are at increased risk for morbidity and mortality from ischaemic heart diseases (IHD) which accounts for almost half of all deaths in RA. Half of the RA patients with confirmed IHD had clinically silent disease. Therefore, early detection of Silent IHD can decrease the cardiovascular mortality in patients with rheumatoid arthritis. **Objectives:** To assess the incidence and identify the predictors of silent ischemic heart disease (SIHD) in patients with rheumatoid arthritis. **Methods:** One hundred eighty patients with rheumatoid arthritis with no history of IHD were studied. All patients subjected to full history taking and full clinical examination and investigated to fasting blood glucose, 2 hour post prandial blood glucose, serum creatinine, mean platelet volume, homocysteine level, urinary microalbuminuria, lipid profile RF, ESR, CRP, Resting ECG and stress ECG. **RESULTS:** Prevalence of silent ischemic heart disease in rheumatoid arthritis patients is 10.6%. Significantly increased incidence of SIHD among patients with rheumatoid arthritis with hypertension (27.9%), peripheral neuropathy (21.1%), microalbuminuria (56.7%) and family history of IHD (28.9%) ( $p$  value < 0.05). Important predictors for SIHD in RA patients were: increased body mass index, increased duration of rheumatoid arthritis, hypertension, increased mean platelet volume, hyperlipidemia, hyperhomocysteinaemia, and high CRP and RF titre. **Conclusions:** Silent IHD is a rather common incidence in rheumatoid arthritis patients (10.6%). The predictors for SIHD are prolonged disease duration, hyperlipidemia, increased mean platelet volume, obesity, hypertension, hyperhomocysteinaemia and presence of activity markers. "Targeting these risk factors in RA patients could help in lowering incidence of ischemic heart disease and its complications".

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**Key Words:** Silent ischaemic heart disease, rheumatoid arthritis.

### 1. Introduction

Rheumatoid arthritis (RA) is one of the most common chronic inflammatory disorders associated with enhanced cardiovascular morbidity and mortality (1). The most common cause of death in RA is cardiovascular disease, accounting for more than 50% of the mortality (2). The most likely explanation is that the inflammation associated with RA has an impact on the vasculature (3).

The pathogenic mechanisms involved in accelerated cardiovascular complications in rheumatoid arthritis appear to be complex and multifactorial. Both traditional and nontraditional risk factors potentially contribute to the increased cardiovascular risk. There is a need for heightened awareness of the increased risk for silent ischemia, early myocardial infarction, and sudden death (4). The underlying cause of ischaemic heart disease (IHD), appears to be accelerated in patients with RA. The reason for this may be related to clustering of classical cardiac risk factors such as dyslipidaemia, a prothrombotic state and other processes. However, classical risk factors, although important, do not appear to be sufficient to explain the accelerated atherosclerosis associated with RA (5). This is possibly

due to the systemic inflammation associated with RA, which may make RA itself (like diabetes) an independent risk factor for the development of IHD (6). Accumulating evidence suggests that systemic inflammation indeed has an important role in the development of atherosclerosis (7) and markers of inflammatory activity such as C reactive protein (CRP) are predictive of cardiovascular risk in the general population (8). A higher risk of sudden cardiac death is associated with particular HLA-DRB1 genotypes that are more frequent in patients with RA. and this can explain in part the higher risk of sudden death in these patients (9).

There is evidence that the presentation of coronary heart disease is different in RA patients compared with individuals without RA. Ischemic heart disease may be clinically silent in many RA patients, and there appears to be a higher risk of unrecognized myocardial infarction and sudden cardiac death. RA patients also have a lower likelihood of demonstrating angina symptoms. Furthermore, the increased risk of coronary heart disease in RA precedes the ACR criteria-based diagnosis of RA, and is not due to an increased incidence of traditional risk factors (10). Enhanced inflammatory process may promote the

development of heart dysfunction in inflammatory arthritis(11).

In seropositive RA, the extent of inflammation has been shown to predict CV disease and overall mortality(9), so aggressive coronary heart disease prevention strategies should be tested for persons with rheumatoid arthritis to decrease mortality(12). A number of predictors for risk of CV disease are well known for the general population, but identification of new markers is still required to help with better characterization of patients at risk of SIHD. This may be particularly important for patients with disability, who, owing to their reduced exercise capacity, may not elicit symptoms of cardiac ischaemia, or whose symptoms may be wrongly attributed to musculoskeletal causes.(13)

## 2. Subjects and methods:

Two hundred patients (aged 35–76 years) with a diagnosis of RA, attending the Menoufiya University hospitals Internal Medicine Clinic, were recruited into a study investigating the prevalence of silent IHD in RA. All patients fulfilled the 2010 American College of Rheumatology criteria for RA. 180 patients completed the full cardiovascular investigation protocol. The study had local research ethics committee approval.

**Inclusion criteria:** Include patients with rheumatoid arthritis aged between 35–60 years.

**Exclusion criteria:** Include patients known to have IHD, DM, severe or malignant hypertension and patients with disabilities which may interfere with stress ECG test as orthopedic or neurological disabilities.

All patients were subjected to full history taking as age, sex, smoking, family history of IHD, family history of Rheumatoid Arthritis or diabetes, RA disease duration and presence of complications. A family history of IHD was defined as a male or female first degree relative sustaining any ischemic cardiovascular disease.

Clinical evaluations Standardized history and examination were performed by a single observer. Cardiovascular symptoms and risk factors were assessed Hypertension was assessed by previous diagnosis, resting systolic and diastolic blood pressure. Height and weight were recorded and body mass index calculated, weight (Kg) divided by the square root of height in meter)..

Cardiovascular investigations: Twelve lead ECG recordings were taken. Detection of cardiac ischaemia was carried out using exercise treadmill stress ECG test.

### Exercise treadmill testing:

All patients were subjected to exercise treadmill stress ECG using the modified Bruce protocol. Heart rate, blood pressure, and a 12-lead electrocardiogram

were obtained at baseline and at each stage of the exercise protocol (every 3 min) . Predicted peak heart rate was calculated as  $220 - \text{age}$  (14). Patients were encouraged to perform a treadmill exercise test until they reached an endpoint. Exercise endpoints included physical exhaustion, significant arrhythmia, severe hypertension (systolic blood pressure >240 mmHg or diastolic blood pressure >110 mmHg), or severe hypotensive response (decrease >20 mmHg in systolic blood pressure from baseline). Ischemic ECG abnormalities during the test were defined as the development of ST-segment deviation of  $\geq 1$  mm which was horizontal or down sloping away from the isoelectric line 80 milliseconds after the J point (15).

Silent ischaemia was defined as ischemia on stress test in the absence of angina and/or ECG changes of either a bundle branch block or ST segment abnormality consistent with IHD (14&15).

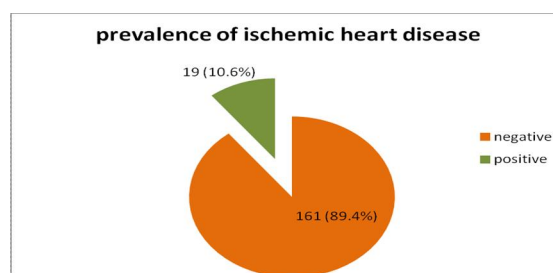
Laboratory investigations including CBC, mean platelet volume, fasting blood glucose, 2 hour post prandial blood glucose, ESR, CRP, RF, homocysteine level, serum creatinine, urinary microalbuminuria and lipid profile.

### Statistical analysis:

Data were analyzed using IBM SPSS version 20. Normality of distribution was computed by kolmogrov smirnov test for numerical variables. Categorical data were expressed in frequencies and percentages while numerical data were expressed in means  $\pm$  SDs . Comparison of quantitative data was performed by Mann Whitney test. Comparison of categorical variables was done using  $\chi^2$  test or Fisher exact test where appropriate. Binary logistic regression was performed for variables associated with ischemic heart disease. Statistical significance was set at 0.05 level.

## 3. Results:

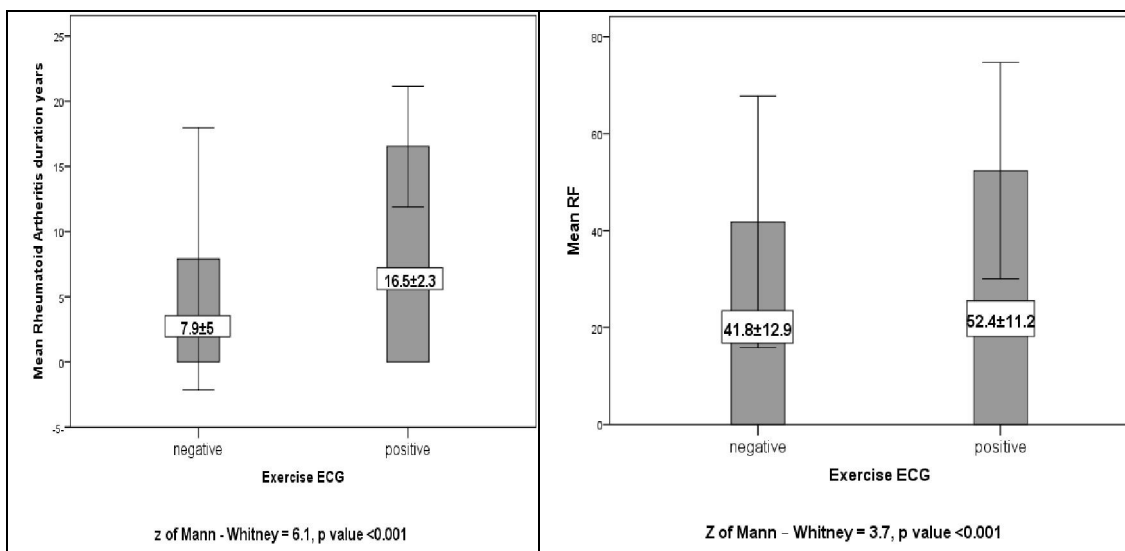
Prevalence of silent ischemic heart disease in rheumatoid arthritis patients in the study is 10.6% as shown in figure (1).



**Figure(1):** Prevalence of silent ischemic heart disease in rheumatoid arthritis patients:

There is significant Association between both increased duration of rheumatoid arthritis and increased rheumatoid factor titre and occurrence of

silent ischemic heart disease in RA patients as shown in figure (2).



**Figure(2):** Association between rheumatoid factor and rheumatoid arthritis duration and silent ischemic heart disease in rheumatoid arthritis patients:

There is significantly increased incidence of silent ischemic heart disease ( $p$  value < 0.05) amongst patients with hypertension (27.9%), peripheral neuropathy (21.1%), presence of

microalbuminuria (56.7%) and family history of ischemic heart disease (28.9%) as shown in table (1).

**Table (1):** Comparison between Ischemic and non-Ischemic group regarding history and laboratory data (categorical variables) in rheumatoid arthritis patients.

History and laboratory data	Exercise ECG(n= 180)				OR (95%CI)	$\chi^2$	p-value
	Positive (n=19)		Negative (n= 161)				
	No	(%)	No	(%)			
<b>Gender:</b>							
Male	11	(14.1)	67	(85.9)	0.5 (0.2, 1.4)	1.8	0.176
Female	8	(7.8)	94	(92.2)			
<b>Smoking:</b>							
Yes	6	(19.4)	25	(80.6)	2.5 (0.8, 7.2)	2	0.104
No	13	(8.7)	136	(91.3)			
<b>Hypertension:</b>							
Yes	19	(27.9)	49	(72.1)	--	34.9	< 0.001**
No	0		112	(100)			
<b>Peripheral neuritis:</b>							
Yes	19	(21.1)	71	(78.9)	--	21.2	< 0.001**
No	0		90	(100)			
<b>Microalbuminuria:</b>							
Yes	17	(56.7)	13	(43.3)	96.7 (20, 465.6)	75.3	< 0.001**
No	2	(1.3)	148	(98.7)			
<b>Family history of IHD:</b>							
Yes	11	(28.9)	27	(71.1)	6.8 (2.5, 18.5)	14.9	< 0.001**
No	8	(5.6)	134	(94.4)			

There are significant association between increase in Age, BMI, RA duration, ESR, CRP, mean platelet volume, total cholesterol, LDL,

creatinine level, homocysteine level and occurrence of silent ischemic heart disease in RA patients. ( $p$  value  $< 0.05$ ) as shown in table (2).

**Table (2):** Comparison between Ischemic and non-Ischemic group regarding history, clinical and laboratory data (continuous variables) in rheumatoid arthritis patients.

	Exercise ECG		Z of Mann – Whitney	p-value
	Positive (Mean± SD)	negative (Mean± SD)		
Age	61.2±3.7	51.3±8.4	4.9	<0.001**
Body Mass Index (kg/m <sup>2</sup> )	27.3 ±2.6	25.4 ± 2.1	3.3	<0.001**
Mean platelet volume(fl):	9.9 ± 0.6	8.2 ± 0.4	6.7	<0.001**
RF	52.4±11.2	41.8±12.9	3.7	<0.001**
Duration of RA	16.5±2.3	7.9 ± 5	6.1	<0.001**
ESR (1st hour):	67.2 ± 13.3	41.6 ± 13.3	5.5	<0.001**
CRP	31.8±5.1	26.8 ± 8.3	3.2	<0.001**
Creatinine	1.9 ± 0.5	0.9 ± 0.3	6.4	<0.001**
Total cholesterol	218.3 ± 37.1	194.5 ± 42.8	2.6	0.010
Triglycerides	168.4 ±25	176.3 ± 40.4	0.8	0.393
LDL	139.7 ± 39.3	112.4 ± 46.3	2.4	0.015*
HDL	43.7± 6.5	45.9 ± 6.5	1.6	0.102
Homocysteine level (µmol/L)	14.9± 1.6	10.9 ± 2.6	5.7	<0.001**

**Table (3):** Logistic regression for important risk factors for silent ischemic heart disease in rheumatoid arthritis patients:

Variables	Regression coefficient	Wald	OR (95%CI)	P-value
Body Mass Index	0.72	8.4	0.5 (0.3, 0.8)	0.004*
Rheumatoid arthritis duration in years	0.26	10.3	1.3 (1.1, 1.5)	0.001**
Rheumatoid factor	0.1	2.7	1.1 (0.9, 1.3)	0.103
Family history of IHD	1.8	3.4	6 (0.8, 40.9)	0.067
Hypertension	4.2	9.1	63.8 (4.3, 949.5)	0.003*
Total cholesterol	0.02	3.9	0.9 (0.9, 1)	0.048*
Mean platelet volume (fl)	1.8	7.8	5.9 (1.7, 20.2)	0.005*
CRP	0.2	3.8	0.8 (0.6, 1)	0.049

Binary logistic regression showed that BMI, hypertension, family history of CAD, duration of RA, total cholesterol, mean platelet volume and inflammatory markers (RF and CRP) are independent predictors for silent ischemic heart disease in rheumatoid arthritis patients.

#### 4. Discussion:

In the current study SIHD was found in 19 patients (representing 10.6 %). *Maradit-Kremers et al., 2005,(10)* concluded that Patients with RA have a significantly higher risk of CHD when compared with non-RA subjects. RA patients are less likely to report symptoms of angina and more likely to experience unrecognized MI and sudden cardiac death. RA has a greater burden of coronary atherosclerosis at their first angiogram that is

independent of traditional CV risk factors. This may be due, at least in part, to the expansion of no classic CD4<sup>+</sup>T cells that have previously been implicated in the pathogenesis of IHD(16). Patients with RA are 30% to 60% more likely to suffer a CV event compared with the general population(17&18), especially myocardial infarction (19&20) .

In our study age was found to have significant association with SIHD in patients with RA. This is consistent with the results of *Cecilia et al., 2006. (21)*

We found insignificant correlation between smoking and SIHD in patients with RA in our study. This finding may be because smoking habit is uncommon in female in our people who represent most of our patients. On the other hand

*Cecilia et al., 2006, (21)* found significant correlation of smoking with IHD in patients with RA. *Chung et al., 2005,(22)* reported that the prevalence and severity of coronary calcification is increased in established rheumatoid arthritis and is related, in part, to smoking.

There was insignificant difference between ischemic and non-ischemic group according to the presence of family history of IHD but this not agree with the results of *Matty et al., 2004 and Chiharu et al., 2006.(5&23)*

In the present study the frequency of SIHD increased in rheumatoid arthritis patients with prolonged duration and this goes hand to hand with the study of *Fietta and Delsante 2009, (24)* who reported that atherosclerosis is an early and common finding in RA patients, positively correlating to the disease duration and severity. On the contrary *Galiutina and Bychak 2011, (25)* found that silent myocardial ischemia in patients with RA have no significant correlation with the disease duration..

Our study also showed a significant association between SIHD in patients with RA and the presence of nephropathy which was diagnosed by the presence of microalbuminuria. *Mpofu and colleagues 2004, (26)*. concluded that microalbuminuria is likely to correlate only poorly with IHD, whether prevalent or silent, in their population of patients with RA (*Kitas and Erbs, 2004*)<sup>(27)</sup> agree that, from the practical perspective, it is very important to identify predictive markers of IHD in RA, and microalbuminuria is a very reasonable candidate for the reasons that *Mpofu and colleagues, (26)* clearly outline. However, they disagree with the conclusion that 'this simple test cannot be used as a surrogate marker for IHD or IHD risk in patients with RA.

Our study also showed that the occurrence of SIHD is significantly increased in patients with RA in the presence of hypertension. *Kitas and Erbs 2004, (27)* have found that more than half (56%) of RA patients with no known cardiovascular co morbidity have hypertension and hypertension should therefore be actively sought and targeted as a risk factor in patients with RA.

Interestingly we found that the occurrence of SIHD is significantly increased in patients with RA in the presence of increased mean platelet volume(MPV) and this agree with *Kilicli et al., 2005,(28)* who found that high MPV is an independent risk factor for coronary atherosclerosis and MI. and also agree with *Gasparyan et al., 2011,(29)* who found that high MPV associates with a variety of established risk

factors, cardio- and cerebrovascular disorders prone to arterial and venous thrombosis.

Our study also showed that the occurrence of SIHD is significantly increased in RA in the presence of increased BMI and this agree with *Stavropoulos et al., 2009,(30)* who found that In RA, strong positive associations have been found between high BMI and adverse CVD risk factors. On the contrary *Kremers et al., 2004* found that among patients with RA, low BMI is associated with a significantly increased SIHD and risk of cardiovascular death.(31) All traditional CV risk factors, except obesity and physical inactivity, were associated with CV morbidity, and in multivariate models, hypertension, hyperlipidemia, diabetes, and ever-smoking remained independent risk factors (32) .

It was noticed that the occurrence of SIHD is significantly increased in RA in the presence of hyperhomocysteinaemia. A high prevalence of hyperhomocysteinaemia in Mexican patients with RA who develop high rates of coronary artery diseases.(33) *Galiutina and Bychak 2011* also found that silent myocardial ischemia in patients with the RA was associated with hyperhomocysteinaemia.(25)

We also found that the occurrence of SIHD is significantly increased in patients with RA in association with the high markers of inflammation and activity as high ESR, increased CRP and high RF titre and this agree with other studies as that of *Chung et al., 2005*, who reported that the prevalence and severity of coronary calcification is increased in established rheumatoid arthritis and is related, in part, to elevated inflammatory markers.(22) *Maradit-Kremers et al., 2005.(10)* confirmed that markers of systemic inflammation confer a statistically significant additional risk for cardiovascular death among patients with rheumatoid arthritis. Systemic inflammation plays an important role in the development of atherosclerosis, and the extent of inflammation in RA patients has been shown to be predictive of cardiovascular disease and overall mortality.(34) *Galiutina and Bychak 2011,(25)* also found that silent myocardial ischemia in patients with the RA was associated with high activity of inflammatory process. CV morbidity and mortality strongly correlate with disease activity, whereas the successful pharmacological control of the chronic inflammation decreases the risk of CV complications.(24) *Myasoedova et al., 2011,(35)* also reported that their findings underscore the importance of systemic inflammation as a key player in the development of CVD in RA by demonstrating independent associations of ESR

and CRP with cardiovascular outcomes and mortality. This is concordant with the concept of acceleration of cardiovascular risk and mortality with increasing inflammatory burden and suggests the need for minimization of cumulative inflammation in RA.(36-38)

Resting ECG has a low sensitivity in detection of SIHD in patients with RA.

#### Conclusion:

- Silent ischaemic heart disease is a rather common incidence in rheumatoid arthritis patients (10.6 %).
- The predictors for SIHD are hyperlipidemia, obesity, hypertension, hyperhomocysteinaemia, increased mean platelet volume, prolonged disease duration and presence of high activity markers. "Targeting these risk factors in RA patients could help in lowering incidence of ischemic heart disease and its complications".

#### Recommendation:

Stress ECG is highly recommended as a better screening method for SIHD especially in the presence of its predictors in patients with RA.

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#### References:

1. **Gasparyan AY, Stavropoulos-Kalinoglou A, Toms TE, Douglas KM and Kitas GD (2010):** Association of mean platelet volume with hypertension in rheumatoid arthritis. *Inflamm Allergy Drug Targets; 9(1): 45-50.*
2. **DeMaria AN(2002):** Relative risk of cardiovascular events in patients with rheumatoid arthritis. *Am J Cardiol.; 89(1): 33-8D.*
3. **Sattar N, McCarey DW, Capell H and McInnes IB(2003):** Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. *Circulation; 108: 2957-63.*
4. **Kaplan and Mariana J (2006):** Cardiovascular disease in rheumatoid arthritis. *Current Opinion in Rheumatology; 18(3): 289-297.*
5. **D L Mathey, P T Dawes, N B Nixon, L Goh, M J Banks and G D Kitas(2004):** Increased levels of antibodies to cytokeratin 18 in patients with rheumatoid arthritis and ischaemic heart disease. *Ann Rheum Dis; 63: 420-425.*
6. **Kitas, GD and Erb, N (2003): Tackling ischaemic heart disease in rheumatoid arthritis. Rheumatology;42: 607-13.**
7. **Ross R(1999):** Atherosclerosis--an inflammatory disease. *N Engl J Med.; 340(2): 115-26.*
8. **Morrow DA and Ridker PM (2000):** C-reactive protein, inflammation, and coronary risk. *Med Clin North Am; 84(1): 149-61.*
9. **Mathey D. L, Thomson W, Ollier W. E. R, Batley M, Davies P. G, Gough A. K, Devlin J, Prouse P, James D. W, Williams P. L, Dixey J, Winfield J, Cox N L, Koduri G and Young A (2007):** Association of DRB1 shared epitope genotypes with early mortality in rheumatoid arthritis: Results of eighteen years of followup from the early rheumatoid arthritis study. *Arthritis & Rheumatism; 56 (5): 1408-16.*
10. **Maradit-Kremers H, Crowson CS, Nicola PJ, Ballman KV, Roger VL and Jacobsen SJ (2005):** Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study. *Arthritis Rheum; 52: 402-11.*
11. **Maradit-Kremers H, Nicola PJ, Crowson CS, Ballman KV, Jacobsen SJ, Roger VL and Gabriel SE (2007):** Raised erythrocyte sedimentation rate signals heart failure in patients with rheumatoid arthritis. *Ann Rheum Dis.; 66(1): 76-80.*
12. **Solomon DH, Elizabeth W. Karlson, Eric B. Rimm, Carolyn C. Cannuscio, Lisa A. Mandl, JoAnn E. Manson, Meir J. Stampfer and Gary C. Curhan (2003):** Cardiovascular Morbidity and Mortality in Women Diagnosed With Rheumatoid Arthritis. *Circulation.; 107: 1303-7.*
13. **Banks MJ, Kitas GD (1999):** Patients' physical disability in RA may influence doctors' perceptions of suitability for risk assessment of CHD. *BMJ; 319: 1266*
14. **Bouzas-Mosquera A, Peteiro J, Alvarez-Garcia N, Brouillon FJ, Mosquera VX, Garcia-Bueno L, Ferro L and Caserto-Beiras A (2009):** Prediction of mortality and major cardiac events by exercise echocardiography in patients with normal exercise electrocardiographic testing. *J Am Coll Cardiol;53:1981-1990.*
15. **Nishime EO, Cole CR, Blackstone EH, Pashkow FJ and Lauer MS (2000):** Heart rate recovery and treadmill exercise score as predictors of mortality in patients referred for



- exercise ECG. *J Am Med Assoc*; 284: 1392-1398.
16. **Kenneth J Warrington, Peter D Kent, Robert L Frye, James F Lymp, Stephen L Kopecky, Jörg J Goronzy and Cornelia M Weyand (2005):** Rheumatoid arthritis is an independent risk factor for multi-vessel coronary artery disease: a case control study. *Arthritis Research & Therapy*; 7(5):984-91.
  17. **Han C, Robinson DW, Hackett MV, Clark P, Fraeman KH and Bala MV(2006):** Cardiovascular disease and risk factors in patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. *J Rheumatol*; 33:2167-72.
  18. **Watson DJ, Rhodes T and Guess HA(2003):** All-cause mortality and vascular events among patients with rheumatoid arthritis, osteoarthritis, or no arthritis in the UK General Practice Research Database. *J Rheumatol*; 30:1196-1202.
  19. **Turesson C, Jarenros A and Jacobsson L(2004):** Increased incidence of cardiovascular disease in patients with rheumatoid arthritis: results from a community based study. *Ann Rheum Dis*; 63: 952-55.
  20. **Wolfe F, Freundlich B and Straus WL(2003):** Increase in cardiovascular and cerebrovascular disease prevalence in rheumatoid arthritis. *J Rheumatol*; 30: 36-40.
  21. **Cecilia P Chung, Annette Oeser, Ingrid Avalos, Tebeb Gebretsadik, Ayumi Shintani, Paolo Raggi, Tuulikki Sokka, Theodore Pincus, and C Michael Stein (2006):** Utility of the Framingham risk score to predict the presence of coronary atherosclerosis in patients with rheumatoid arthritis. *Arthritis Res Ther*; 8(6): R186.
  22. **Chung CP, Oeser A, Raggi P, Gebretsadik T, Shintani AK, Sokka T, Pincus T, Avalos I and Stein CM (2005):** Increased coronary-artery atherosclerosis in rheumatoid arthritis: relationship to disease duration and cardiovascular risk factors. *Arthritis Rheum*; 52: 3045-53.
  23. **Chiharu Kishimoto, Miki Hirata, Kaori Hama, Masami Tanaka, Kazushi Nishimura, Shigeru Kubo, Kinzo Ueda, Tatsuo Fujioka and Shunichi Tamakil(2006):** Carotid intima-media thickness is increased in subjects with ischemic heart disease having a familial incidence. *Exp Clin Cardiol*; 11(1): 8-10.
  24. **Fietta P and Delsante G (2009):** Atherogenesis in rheumatoid arthritis: the "rheumatoid vasculopathy"? *Acta Biomed*; 80(3): 177-86.
  25. **Galiutina OIU and Bychak OV (2011):** Relationship of silent myocardial ischemia with the course of rheumatoid arthritis and hyperhomocysteinemia. *Lik Sprava*; 1(2):48-52.
  26. **Mpofu S, Kaushik VV, Grundy G and Moots RJ(2004):** Microalbuminuria: is it a predictor of ischaemic heart disease in rheumatoid arthritis? *Rheumatology*; 43 (4): 537-538.
  27. **Kitas GD and Erbs N(2004):** Reply: Microalbuminuria: is it a predictor of ischaemic heart disease in rheumatoid arthritis? *Rheumatology (2004)* 43 (4): 538.
  28. **Kiliçli-Camur N, Demirtunç R, Konuralp C, Eskiser A and Başaran Y(2005):** Could mean platelet volume be a predictive marker for acute myocardial infarction? *Med Sci Monit*; 11(8):387-92.
  29. **Gasparyan AY, Ayyazyan L, Mikhailidis DP, Kitas GD (2011):** Mean platelet volume: a link between thrombosis and inflammation? *Curr Pharm Des*; 17(1): 47-58.
  30. **Stavropoulos-Kalinoglou A, Metsios GS, Panoulas VF, Douglas KM, Nevill AM, Jamurtas AZ, Kita M, Koutedakis Y and Kitas GD (2009):** Associations of obesity with modifiable risk factors for the development of cardiovascular disease in patients with rheumatoid arthritis. *Ann. Rheum. Dis*. 68, 242-45.
  31. **Kremers HM, Nicola PJ, Crowson CS, Ballman KV, Gabriel SE (2004):** Prognostic importance of low body mass index in relation to cardiovascular mortality in rheumatoid arthritis. *Arthritis Rheum*; 50(11): 3450-7.
  32. **Antonio Naranjo, Tuulikki Sokka, Miguel A Descalzo, Jaime Calvo-Alén and Kim Hørslev-Petersen(2008):** Cardiovascular disease in patients with rheumatoid arthritis: results from the QUEST-RA study. *Arthritis Research & Therapy*; 10(2):R30.
  33. **Lopez Olive MA, Gonzalez-Lopez L, Garcia Gonzalez A, Villa-Manzano AI, Cota Sanchez AR, Salazar Paramo M, Varon Villalpando F, Cardona-Munoz EG and Gamez-Nava JI (2006):** Factors associated with hyperhomocysteinemia in Mexican patients with rheumatoid arthritis. *Scand J Rheumatol*; 35(2): 112-6.

34. **Del Rincon I, Freeman GL, Haas RW, O'Leary DH and Escalante A (2005):** Relative contribution of cardiovascular risk factors and rheumatoid arthritis clinical manifestations to atherosclerosis. *Arthritis Rheum*; 52: 3413–23.
35. **Elena Myasoedova, Cynthia S. Crowson, Hilal Maradit Kremers, Veronique L. Roger, Patrick D. Fitz-Gibbon, Terry M. Therneau, and Sherine E. Gabriel (2011):** Lipid paradox in rheumatoid arthritis the impact of serum lipid measures and systemic inflammation on the risk of cardiovascular disease. *Ann Rheum Dis.*; 70(3): 482–487.
36. **Gabriel SE (2008):** Why do people with rheumatoid arthritis still die prematurely? *Ann Rheum Dis.*; 67( 3): 30–4.
37. **Avina-Zubieta JA, Choi HK and Sadatsafavi M (2008):** Risk of cardiovascular mortality in patients with rheumatoid arthritis: a meta-analysis of observational studies. *Arthritis Rheum.* 2008;59(12):1690–7.
38. **Kiely PD, Brown AK and Edwards CJ (2008):** Contemporary treatment principles for early rheumatoid arthritis: a consensus statement. *Rheumatology (Oxford)*; 48(7): 765–72.

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## 16S rRNA gene-based Bacterial Community in Polychlorinated Biphenyls (PCBs) contaminated site using PCR- Single-Strand Conformation Polymorphism (SSCP)

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**Abstract:** The oxidation pond site in Sadat city, Egypt had been polluted with PCBs compounds more than a decade ago because of the wastes collected from different manufactures. Culture-independent approach generate a more accurate view for the bacterial community of the PCBs contaminated site. The diversity of bacterial populations in site contaminated with polychlorinated biphenyls (PCBs) was investigated using Cultivation-independent technique PCR-based single-strand conformation polymorphism (SSCP) for genetic profiling of PCR-amplified 16S rRNA genes. SSCP was performed using Com primer set targeting the 16S rRNA genes. The SSCP analysis showed increasing of the microbial communities from phyla *Proteobacteria* and certainly in the family *Sphingomonadaceae* correlated with increasing of PCBs in the contaminant site. This finding may benefit as an effective tool for bioremediation by facilitating the detection and monitoring of the PCBs degrader with SSCP technique.

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**Keywords:** Bacterial community; Culture-independent; PCR-SSCP; Polychlorinated Biphenyls (PCBs)

### 1. Introduction

Sadat City is one of the largest industrial city in Egypt, located in the west of Al-Menufiya Province, at 93 km from Cairo. Area: 500 km<sup>2</sup>, Population: 900,200. It is a desert area and includes more than 109 factories with several industrial activity such as iron-steel industry, paints, ceramic, chemicals, foods, fertilizers, biocides, organic products, textile, paper, batteries, dyes, printing materials and recycled plastics. The final outputs of industrial as well as urban effluents are expelling out of the populated area of the city. The treatment of such emissions is carried out depending on the oxidation pond system (Elaxadaponds). However, the sweeping overflow of this discharge move downward to the lower land forming large pools (2.6-4 hectares, 2-4 m depth). The Oxidation Pond site in Sadat city, Egypt had been polluted with PCBs compounds.

PCBs are man-made chemicals and synthesized by substituting variable number of chlorine atoms (from 1 to 10 chlorine atoms) onto the biphenyl aromatic molecular structure to produce 209 congeners. These congeners were used widely as coolant in power transformers, insulators in capacitors, heat transfer fluids, fire resistance, and plasticizers as well as in consumer products such as ink, paper and paints. PCBs are a main category of persistent organic pollutants (POPs) present as contaminants in the environment (IPCS, 1993; 1997). Exposure to elevated levels of PCBs has caused birth defects and cancer in laboratory animals, and they are a suspected cause of cancer and adverse skin and liver effects in humans (USEPA, 2008).

Cultivation of microorganisms isolated from PCBs contaminated sites or any variety of ecosystem samples by any chosen cultivation approach will inevitably favor the growth of some community members while others are inhibited or not cultured and allows only around 0.1 to 1% of the total viable bacterial cells present in a variety of ecosystems can be cultured (Amann *et al.*, 1995). It is unlikely that any cultivation method will not allow a full description of the microbial diversity. Therefore, adapting or developing dedicated molecular methods for the culture-independent survey of microorganisms take advantage producing complete information for PCBs contaminated sites.

A developed protocol was used, which allows the application of single-strand-conformation polymorphism (SSCP) (Orita *et al.*, 1989; Hayashi, 1991) for the culture-independent assessment of microbial-community diversity (Schwieger and Tebbe, 1998; Jean and Georges, 2008; Keskes *et al.*, 2012). The SSCP method has the potential to be more easily applied (Lee *et al.*, 1996) and the SSCP produced a number of sharp bands and differentiated the bacterial community structures (Tomoyuki Hori *et al.*, 2006). SSCP was optimized to analyze only one of the complementary single strands (Schwieger and Tebbe, 1998; Meng-zhi *et al.*, 2008), by preferentially degrading with lambda exonuclease the one strand generated with a phosphorylated primer. This development aims to avoid heteroduplex formations, or overlapping of forward-reverse strands from different amplicons during separation, allowing the separation of mixtures of fragments of identical size

but different in sequence. The application of this modified technique was focused on studies of taxonomic shifts in microbial communities by targeting 16S rRNA genes (Peters *et al.*, 2000; Schwieger and Tebbe, 2000; Schmalenberger *et al.*, 2001; Schmalenberger and Tebbe, 2003). However, a potential application to assess diversity of functional genes was foreseen (Stach and Burns, 2002).

This study tries to identify the bacterial community in the Oxidation pond (Elaxada-ponds) in Sadat City as one of PCBs contaminated site by culture independent method via SSCP

## 2. Material and Methods

### DNA Extraction from the four soil samples.

Total DNA from the Four PCBs-contaminated soil samples collected from the different treatments stages of the oxidation pond which contain all the final outputs of 109 factories The soil samples were stored at 4°C until use within 7 days. DNA were then extracted according to the protocol for DNA extraction with the Fast prep DNA kit for soil (Bio 101). DNA was visualized on 1% agarose gels. Yield of genomic DNA was determined spectrophotometrically by measuring the absorbance at 260 nm. Purity was determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm (a pure DNA has an  $A_{260}/A_{280} \sim 2.0$ ) The DNA extracts from PCBs-contaminated samples containing approximately 200 ng ml<sup>-1</sup> DNA were 50- or 100-fold diluted in Tris-HCl buffer (10 mM, pH 8.0) and used as template DNA in PCR.

### SSCP for the four soil samples.

Com1 (5' CAGCAGCCGCGTAATAC3') targeting the position (519-536) and Com2-Ph (5' CCGTCA ATTCCTTTGAGTTT3') targeting the position (907-926) were chosen for the amplification of bacterial 16S rRNA genes (Schwieger and Tebbe 1998). Single-stranded DNA (ssDNA) from PCR products was obtained as previously described (Schwieger and Tebbe, 1998). Briefly, PCR has performed with one of the primers being 5' phosphorylated, PCR products were eluted from agarose gels (Figure 1), and the phosphorylated strands were digested by lambda exonuclease (NEB). The remaining single-strands were purified with Qiaquick PCR Cleaning Kit (Qiagen), dried by vacuum centrifugation, resuspended in 6µl of loading buffer (95% formamide, 0.25% bromophenol blue and 0.25% xylene cyanol), and denatured for 5 minutes at 94 °C, followed by instant cooling on water ice bath for 3 minutes. The separation conditions were standardized in a DCode System for PCR-SSCP, optimized running parameters were 120V (10 mA) for 18 h at a constant temperature of 26°C on 20 cm x 20 cm x 0,75 mm 0.6X MDE gels in 0.7X TBE (Sambrook *et al.*, 1989) as a running buffer. Optimal

results were obtained when ssDNA obtained from 100 - 400 ng dsDNA was loaded onto the gels and a slightly enhanced resolution was achieved when the amplified single-strands of the reverse primer were subjected to PCR-SSCP analysis. For nucleic acid detection, gels were silver stained as reported previously (Bassam *et al.*, 1991). Single-strand electrophoretic mobilities corresponding to different conformations were excised from dried gels (Figure 2), and DNA extracted by the "Crush and Soak" method (Sambrook *et al.*, 1989) PCR reamplification of the excised and eluted single-strands was made with the same primers used to generate the original dsDNA fragment (Figure 3).

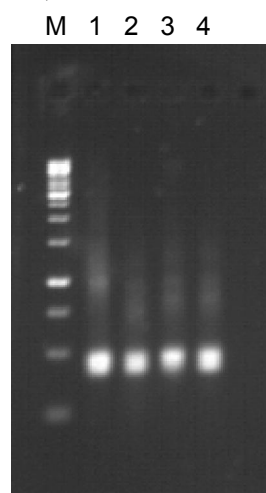


Figure 1 PCR with com primers targeting the 16SrRNA genes for the four DNA extracted samples from the four soil samples collected from the Oxidation-ponds (lanes 1, 2, 3, 4) and M is 1Kb DNA Ladder GeneRuler™, Fermentas

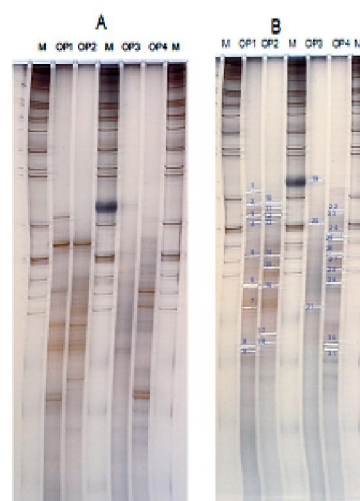


Figure 2 (A) show SSCP on a polyacrylamide gel for the four different soil samples before cutting the bands, (B) after cutting the bands.

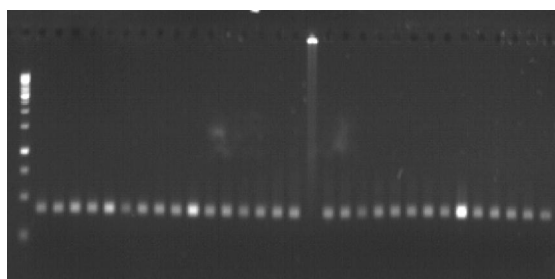


Figure 3. PCR reamplification of the excised and eluted single-strands with the same com primers to generate the original dsDNA fragment lanes 1-31 are the product of the reamplification, lane 17 with the missed product, M is 1Kb DNA Ladder GeneRuler™, Fermentas

### Data deposition

The sequence reported in this study has been deposited in the GenBank database (29 sequences for the independent culture bacteria accession numbers HQ829977- HQ830005).

### 3. Results

Com Primer set were used to amplify the eubacterial 16S rRNA gene sequences including the variable regions yielded complex SSCP patterns, SSCP community profiling showed highly diverse and

distinct microbial communities for the soil samples, the OP-4 soil sample displayed the largest numbers of bands, while sample 3 (OP-3) displayed less of them (Figure 2). The SSCP profiles for the four PCBs contaminated soil shown in Figure.2. By PCR, the opposite strands were regenerated and the products were reamplified. SSCP gel electrophoresis was used to evaluate the purities and identities of the reamplified 31 products, as shown for products obtained from PCR targeting the hypervariable samples 16S rRNA genes only 30 were appeared (Figure. 3). In most cases, reamplification products corresponded to the expected positions in the community patterns and no additional products were observed. These products were then directly used for cloning and DNA sequencing. To identify the predominant products by DNA sequencing, a total of 30 different DNA single strands ("bands") were excised, only 29 sequences were in good appearance. By comparing the 29 sequences Acc. Nr. from HQ829977 to HQ830005 with the related taxa, revealed 18 different operational taxonomic units OTUs of bacteria, and that the compositions of the communities of the four soil samples were all common. These OTUs were most closely related to *Sphingomonas* sp. and *Pseudomonas* sp (Table 1).

Table (1) Phylogenetic assignment of sequences of prominent bands in SSCP gel profiles of the four soil samples communities.

OTU# & Acc. No.	Most closest related to	Similarity (%)	Bp	Acc. No.
1 (HQ829977)	<i>Sphingomonas</i> sp. Ens32	76	358	DQ339627
2 (HQ829978)	<i>Sphingomonas faeni</i> (T); MA-olki	93	396	AJ429239
3 (HQ829979)	uncultured alpha proteobacterium, ATB-LH-6119	91	399	FJ535117
4 (HQ829980)	uncultured bacterium; 4_G07	97	386	FN421770
5 (HQ829981)	uncultured bacterium; 4_H07	91	391	FN421778
6 (HQ829982)	uncultured bacterium; 10_D09	95	396	FN421920
7 (HQ829983)	uncultured alpha proteobacterium; ATB-LH-6119	93	393	FJ535117
8 (HQ829984)	uncultured bacterium; 1_C07	96	385	FN421584
9 (HQ829985)	<i>Pseudomonas</i> sp. TSBY-92	93	392	DQ173037
10 (HQ829986)	<i>Sphingomonas</i> sp. J05	97	390	AJ864842
11 (HQ829987)	uncultured bacterium; 5_H07	97	395	FN421995
12 (HQ829988)	uncultured <i>Sphingomonas</i> sp.; 437D	96	391	AY571827
13 (HQ829989)	uncultured bacterium; BF0002B019	97	387	AM697069
14 (HQ829990)	uncultured bacterium; 3_H05	96	391	FN421710
15 (HQ829991)	uncultured bacterium; 4_H07	97	382	FN421778
16 (HQ829992)	uncultured bacterium; 4_H03	96	384	FN421776
17 (HQ829993)	uncultured bacterium; BF0001B024	97	387	AM696998
18 (HQ829994)	uncultured bacterium; BF0002B019	98	381	AM697069
19 (HQ829995)	uncultured bacterium; 4_H07	55	351	FN421778
20 (HQ829996)	uncultured bacterium; 2_E05	96	353	FN421872
21 (HQ829997)	uncultured <i>Sphingomonas</i> sp.; O11	96	386	AM691108
22 (HQ829998)	uncultured bacterium; BF0002B019	96	387	AM697069
23 (HQ829999)	uncultured bacterium; BF0002B019	97	388	AM697069
24 (HQ830000)	<i>Sphingomonas</i> sp. clone jing-G-41	97	383	HM123760
25 (HQ830001)	<i>Sphingomonas faeni</i> (T); MA-olki	95	390	AJ429239
26 (HQ830002)	uncultured alpha proteobacterium; ATB-LH-6119	98	385	FJ535117
27 (HQ830003)	uncultured alpha proteobacterium; ATB-LH-6119	98	384	FJ535117
28 (HQ830004)	uncultured alpha proteobacterium; ATB-LH-6119;	97	388	FJ535117
29 (HQ830005)	uncultured alpha proteobacterium; ATB-LH-6119;	96	388	FJ535117

The phylogenetic tree (Figure. 4) constructed from the partial sequences of the 16S rRNA amplicons from the 4 soil samples showed a predominance of *Alphaproteobacteria*, and the SSCP bands (OP1-1, OP1-7, OP1-9, OP2-10, OP3-20, OP4-24, OP4-26, OP4-27, OP4-28, OP4-29) were far from any closet published 16S rRNA in the public database (Figure 4), OP1-4 band is very close to Uncultured bacterium partial 16S rRNA gene, clone 4\_H03( Acc. No. FN421776) from phyllosphere of soybean in Switzerland. P4-14 band is very close to the partial sequence of the 16S rRNA of *Pseudomonas* sp. TSBY-92 (Acc. No. DQ173037) from frozen soil in China. Bands no OP2-12 and OP2-16 are very close to Uncultured bacterium partial 16S rRNA gene clone 10- D09 (Acc. No. FN421920) and Uncultured bacterium partial 16S rRNA gene clone 10- H07 (Acc. No. FN421995) from the phyllosphere of clover in Switzerland, and also Bands no. OP1-2 and OP4-22 are very close to many 16S rRNA from the phyllosphere of soybean and clover plants in Switzerland. Band no. OP4-23 is very close to Uncultured *Shingomonas* sp. 16S rRNA gene, isolate O11 (Acc. No. AM691108). Band no. OP4-18 is very close to *Shingomonas faenia* partial 16S rRNA gene, strain MA-olki (Acc. No. AJ429239) (Figure. 2C).

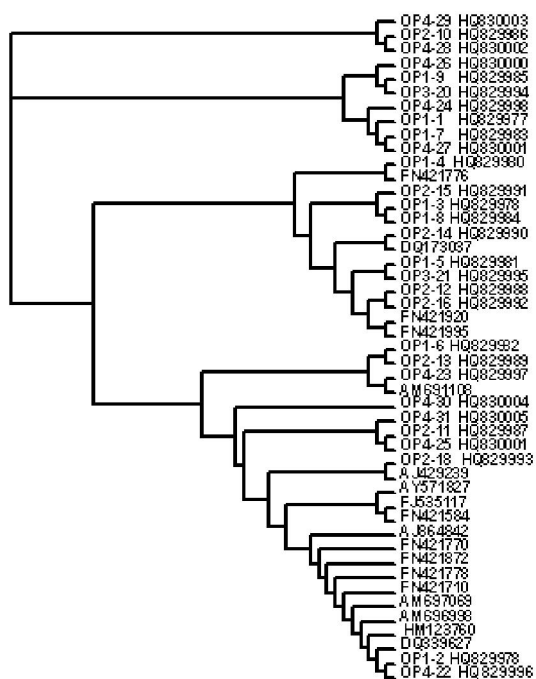


Figure 4 Phylogenetic tree of partial 16S rRNA gene sequences for the SSCP bands and its high similarity

#### 4. Discussions

Soil microorganisms play important roles in maintaining soil quality and ecosystem health. Development of effective methods to analysis

bacterial community structures and encourages the growth of selected microorganisms that are capable of transforming PCBs are challenges for successful bioremediation of PCBs- Contaminated soils.

The cultivation independent methods, based on amplification of environmental DNA followed by acrylamide gel electrophoresis, separate sequence specific DNA fragments of the same length, have the potential for accurate comparison of environmental samples in a short period of time. In this study, we have shown that SSCP analysis of 16S rRNA genes amplified from directly extracted DNA from soil samples can be used to visualize such community structures of highly PCBs contaminated sites, and also this indicate the high potential of this technique to monitor microbial communities and their variation qualitatively and quantitatively (Peters *et al.*, 2000).

Soil sample OP.4 displayed the largest bands than the other bands maybe possibly due to the slightly higher organic carbon content in this sample and this emphasis that the higher chlorinated PCBs provide the organisms neither with energy nor with carbon to support the degradation process and the microbial degradation of PCBs in the environment is influenced by various biological, chemical and physical factors as well as the survival of microorganisms in cases where bioaugmentation is the application of choice (Blumenroth and Wagner-Döbler, 1998; Barriault *et al.*, 1999; Ahn *et al.*, 2001).

As more gene sequences become available, PCR-SSCP-mediated monitoring of different subgroups or microorganisms, due to optimized primer design, will become even more attractive, almost all of these OTUs from the indirect culture (Table 1) in this study are belonging to the phyla *Proteobacteria*. and certainly in the family *Shingomonadaceae* which includes a large number of strains, which have been reported to be capable of degrading and assimilating PCBs and dioxin like compounds via dioxygenation (Akira, 2003).

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#### References

1. Akira H. Biodiversity of dioxin-degrading microorganisms and potential utilization in

- bioremediation. *Microbes Environ* 2003;18: 105-125.
2. Ahn YB, Beaudette LA, Lee H, Trevors JT. Survival of a GFP-labeled polychlorinated biphenyls degrading psychrotolerant *Pseudomonas* sp. In 4 and 22 degrees C soil microcosms. *Microbial Ecol.* 2001;42:614-623
  3. Amann R.I., W. Ludwig, and K.H. Schleifer. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol. Rev.* 1995;59: 143-169.
  4. Barriault D., Vedadi M., Powlowski J. & Sylvestre M. cis-2,3-dihydroxybiphenyl dehydrogenase and cis-1,2-dihydroxynaphthalene dehydrogenase catalyze dehydrogenation of the same range of substrates. *Biochem Biophys Res Commun.* 1999;260:181-187.
  5. Bassam B. J., Caetano-Anolles G., and Gresshoff P. M. Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem.* 1991;196: 80-83.
  6. Blumenroth P., Wagner-Dobler, I. Survival of inoculants in polluted sediments: effect of strain origin and carbon source competition. *Microb. Ecol.* 1998;35:279-288
  7. Hayashi, K. PCR-SSCP: a simple and sensitive method for detection of mutations in the genomic DNA. *PCR Methods Appl.* 1991;1:34-38.
  8. IPCS. Environmental health criteria 140. Polychlorinated biphenyls and tetraphenyls. WHO, Geneva. 1993.
  9. IPCS. Environmental health criteria 195. Hexachlorobenzene, WHO, Geneva. 1997
  10. Jean-Luc Jany and Georges Barbier. Culture-Independent Methods for Identifying Microbial Communities in Cheese. *Food Microbiology*, 2008;25: 839-848
  11. Keskes S, Hmaied F, Gannoun H, Bouallagui H, Godon JJ, Hamdi M. Performance of a submerged membrane bioreactor for the aerobic treatment of abattoir wastewater. *Bioresour Technol.* 2012;103:28-34.
  12. Lee D.-H., Y.-G. Zu, and S.-J. Kim. Nonradioactive method to study genetic profiles of natural bacterial communities by PCR-single strand conformation polymorphism. *Appl. Environ. Microbiol.* 1996;62:3112-3120.
  13. Meng-zhi WANG, Hong-rong WANG, Heng-chun CAO, Guo-xiang LI and Jie ZHANG. Effects of Limiting Amino Acids on Rumen Fermentation and Microbial Community. *In vitro. Agricultural Sciences in China* 2008;7: 1524-1531.
  14. Orita M., H. Iwahana, H. Kanazawa, K. Hayashi, and T. Sekyia. Detection of polymorphisms of human DNA by gel electrophoresis as single-strand conformation polymorphisms. *Proc. Nat. Acad. Sci. USA.* 1989;86:2766-2770.
  15. Peters S., S.Koschinsky, F. Schwieger, and C.C. Tebbe. Succession of microbial communities during hot composting as detected by PCR-single-strand-conformation polymorphism-based genetic profiles of small-subunit rRNA genes. *Appl Environ Microbiol.* 2000;66:930-936.
  16. Sambrook J., Fritsch, E. F., Maniatis, T. *Molecular cloning: a laboratory manual*. Second edition Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, USA. 1989.
  17. Schmalenberger A., F. Schwieger, and C.C. Tebbe. Effect of primers hybridizing to different evolutionarily conserved regions of the small-subunit rRNA gene in PCR-based microbial community analyses and genetic profiling. *Appl Environ Microbiol.* 2000;67:3557-3563.
  18. Schmalenberger A. and C.C. Tebbe. Bacterial diversity in maize rhizospheres: conclusions on the use of genetic profiles based on PCR-amplified partial small subunit rRNA genes in ecological studies. *Mol Ecol.* 2003;12:251-262.
  19. Schwieger F., and C. C. Tebbe. A new approach to utilize PCR-single-strand-conformation polymorphism for 16S rRNA gene-based microbial community analysis. *Appl. Environ. Microbiol.* 1998;64:4870-4876.
  20. Schwieger F. and C.C. Tebbe. Effect of field of inoculation with *Sinorhizobium meliloti* L33 on the composition of bacterial communities in rhizospheres of a target plant (*Medicago sativa*) and a non-target plant (*Chenopodium album*)-linking of 16S rRNA gene-based single-strand conformation polymorphism community profiles to the diversity of cultivated bacteria. *Appl Environ Microbiol.* 2000;66:3556-3565.
  21. Stach J. E. M. and R.G. Burns. Enrichment versus biofilm culture: a functional and phylogenetic comparison of polycyclic aromatic hydrocarbon-degrading microbial communities. *Environ Microbiol.* 2002;4:169-182.
  22. Tomoyuki H., H. Shin, U. Yoshiyuki, I. Masaharu and I. Yasuo. Direct comparison of single-strand conformation polymorphism (SSCP) and denaturing gradient gel electrophoresis (DGGE) to characterize a microbial community on the basis of 16S rRNA gene fragments. *Journal of Microbiological Methods.* 2006;66: 165-169.
  23. U.S. Environmental Protection Agency. "Polychlorinated Biphenyls (PCBs) U.S. EPA, Toxicity and Exposure Assessment for Children's Health teach database at: <http://www.epa.gov/teach>. 2008.

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## Role Of Diode Laser In Preservation Of The Marginal Bone Around Early Loaded Endosseous Implant

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**Abstract:** **Aim:** Evaluation of the effect of diode laser irradiation on crestal bone preservation around early loaded dental implants (used for single tooth replacement) clinically and radiographically. **Patients and Methods:** Eight patients need a bilateral implant placement were included in this study. Implant placed in one side was exposed to diode laser immediately; 4 days and 7 days after insertion of the implants. The other side was not exposed to the laser. The implants were loaded for 6-8 weeks. The patients were followed up clinically and radiographically at time of abutment placement and then at three, and six months. **Results:** Minimal amount of marginal bone resorption around the implants was noticed in the lased side more than in the non-lased side. **Conclusions:** The application of the diode laser to the endosseous implant can preserve the bone around the implant and may aid in improving the longevity of the implants.

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**Key words:** diode laser, implant, marginal bone loss, early loading.

### 1. Introduction:

Nowadays, laser has been widely used in medical and dental applications. The laser used in dentistry included lasers with high power density commonly known as high-level lasers; that are commonly used to produce a thermally destructive effect and selective photocoagulation. The other class of laser is known as low-level lasers or described as therapeutic lasers. The therapy performed with such lasers is often called low-level laser therapy (LLLT), Kahraman(2004). In dentistry, LLLT is effectively used to accelerate healing or improving recovery in cases of aphthous ulcers, mucositis, traumatic ulcers, herpetic lesions, Colvard and Kuo (1991); Lima et al.(2010), nerve injuries, Midamba and Haanaes (1993) and treatment of temporomandibular joint disorders, Mazzetto et al.(2010).

For acceleration or improving the healing of the bone tissue, LLL has been tried in different situations. In orthodontic field, LLL was investigated to accelerate healing and to decrease recurrence after palatal expansion and a good results was obtained, Saito and Shimizu(1997). In oral and maxillofacial surgical procedures, low level laser has been tested to enhance bone growth both in alveolar bone healing after tooth extraction and in bone fracture healing, Takeda (1988); Luger et al.(1998). Trell and Mayayo(1987) conducted an experimental study and showed that low level laser irradiation can speed up vascularization and increase the number of trabeculae in fractured mouse tibiae. In another study, it was found that low level laser therapy significantly

increased the count and activity of osteoblasts, Dortbudak et al.(2000).

In dentistry, the use of dental implants for tooth replacement became state of the art in dental prosthetic therapy. The success of the endosseous dental implant depends largely on the osseointegration of the implant in the bone. Several treatments have been proposed to improve and accelerate bone formation onto implant surface, among which low level laser therapy, Petri et al.(2010).

The studies on use of low level laser to stimulate the osseointegration of the implant are few especially the clinical studies. Two recent studies conducted to evaluate the effect of low intensity laser irradiation on immediately loaded implant supporting over denture. The studies showed the alveolar bone height was preserved while the bone density was increased in the side exposed to low level laser and depending on the good results obtained, the researchers concluded that application of laser to immediately loaded implants preserve the supporting alveolar bone and increase bone density compared to the unlased implants, El-Talawy et al.(2011); Rizk and El-din(2011).

In another study, the effect of diode laser application on osseointegration of dental implant was evaluated and it was found that low level laser application had stimulated bone formation and maturation around the implants, Ismaeel and Abbas(2011). Lastly, El-Desouky et al.(2007) assessed the effect of low level laser irradiation on bone density following loading of previously lased implant after insertion and they found that the bone



density increased more when the implants were lased before and after their loading thus positively affecting the longevity of those implants.

In Alice et al.,(2010) study to investigate the effects of low level laser by application of diode laser on human osteoblastic cell grown on titanium discs, they found that LLLT stimulates the expression of osteoblastic phenotype in cells cultured on titanium suggesting possible benefits on implant osseointegration despite a transient deleterious effect immediately after laser irradiation. In another experimental study conducted by El-Din et al.,(2008) to assess effect of short term administration of vitamin C, associated with low level laser irradiation of the implants, They found that, bone osseointegration had been enhanced when laser irradiation was used. Researchers expected that laser application would reduce healing time and speed up osseointegration of the implants, Dortbudak et al.,(2002).

## 2. Materials and Methods

### 2.1. Materials:

#### 2.1.1. Studied subjects:

Eight patients (7 females and one male) free from any systemic disease, with age range from 27 to 40 years, were selected to participate in this study. Insertion of implants in both sides of the patient's jaws was a part of the treatment plan of those patients.

#### 2.1.2. Implants

The implants used in this study were root form implant (Root form implant, Vitan, France). A total of 16 implants were inserted in the patient's mandible. They were placed in the premolar-molar area of their mandible.

### 2.2. Methods:

#### 2.2.1. Study design:

The technique was 2 stage surgery technique and all the patients were managed under local anaesthesia in the IbinSin dental college, Jedhha, Saudia Arabia by the same surgeon and according to the recommendation of implant's manufacture. After insertion of the implants, one side was ascertained to be the study side and the other served as control.

Laser irradiation was done to the selected side immediately; 4<sup>th</sup> day and 7<sup>th</sup> day after implant insertion. The total time of laser application was 2 minutes each session divided equally on buccal, occlusal and lingual aspect of the implant site. The laser used in this study was 970 nm diode laser (SIROLaser, Sirona dental systems GmbH, Germany). The power was adjusted using the manual mode to be 0.6 watt and frequency was 75 Hertz. The laser delivered to the area by an optic fiber 200 Um in non contact mode. After 6-8 weeks of healing, the implants were exposed and the prosthetic part completed. Fig 1 shows the device used in this study.

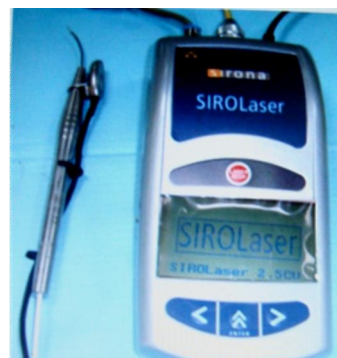


Fig 1 shows the device used in this study

#### 2.2.2. Clinical follow up

The clinical evaluation included two parameters. Loose and lost implants were scored any time after placement and probing depth was measured at four sites of each implant (mesially, Distally, Buccally, and lingually) by using a periodontal probe (Merit B, Hu Friedy, Chicago, IL, USA).

#### 2.2.3. Radiographic follow up

To evaluate the amount of marginal bone loss, a combination of panoramic views and standard periapical radiographs were taken at abutment connection and then at three, and six months after. The change in the crestal bone level on the mesial and distal aspects of the implant was evaluated on the images of the periapical films by using the Adobe Photoshop extended software, where the distance from the implant abutment interface to the level of bone contact with the implant was determined. The data were presented as mean and standard deviation (SD) values and the significance level was set at  $P \leq 0.05$ .

## 3. Results

In this study, eight patients received a total of 16 implants in the premolar-molar region of the mandible. In each patient, one implant was exposed to laser irradiation immediately, at the 4<sup>th</sup> day and at 7<sup>th</sup> day after implant placement. To evaluate the effect of the laser irradiation on the preservation of the marginal bone around the implants, the patients were followed up radiographically to determine the amount of marginal bone loss. For the implants that were exposed to laser, the mean of marginal bone loss was 0.19 mm at 3 months, and 0.35 mm at 6 months. For the implants in the other side, which did not exposed to any laser, the mean of marginal bone loss was 0.45 mm at 3 months and 0.62 mm at 6 months.

In addition, statistical significant difference was found between the MBL in the two sides, the p. value was 0.04 at 3 months and 0.02 at 6 months.

Regarding the peri-implant probing depth, there was no significant difference between the two sides. The probing depth ranged from 2-2.5 mm with a mean of 2.2 mm in the side exposed to laser and for the other

side it ranged from 3-3.5 mm with a mean of 3.27 mm at 3 months. At 6 months, the mean of probing depth was 1.22 mm for the study side and 2.44 mm for the

control side. No implants in the study side or control side were lost or become loose.

**Table (1): The mean and standard deviation of the marginal bone loss and probing depth as a function of time for both sides (study and control sides)**

Time/Side		Marginal bone loss			Probing depth		
		Mean	SD	P. Value	Mean	SD	P. value
At 3 months	<i>Study side</i>	0.19	4.183	0.04	2.22	0.259	1.000
	<i>Control side</i>	0.45	0.112		3.27	0.288	
At 6 months	<i>Study side</i>	0.35	3.536	0.02	1.22	0.228	0.24
	<i>Control side</i>	0.62	0.110		2.44	0.329	

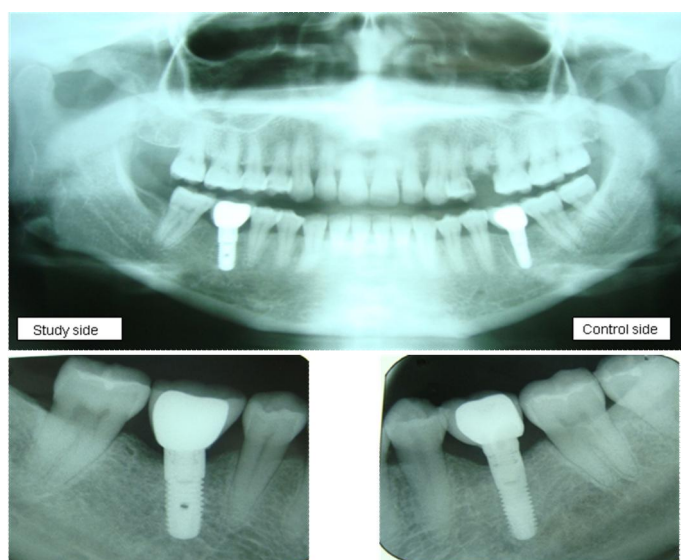


Fig 2 the panoramic view done at insertion of the crowns and the periapical radiographs at 6 months after delivery of the crowns for one case.

#### 4. Discussion:

Mester et al., mentioned in (1971) the first report concerning biological stimulation of lasers and whether low level laser therapy accelerates the ossification or not. There have been several reports that use of a low level laser after implant insertion promoted osteointegration due to rapid bone turn over. Diode laser has excellent transmission efficiency to tissue compared to other laser systems. This laser has been used to promote ossification. Low level laser therapy had been reported to reduce the period of bone fracture healing, Khadra et al., (2004); Khadra et al., (2005).

In the implant dentistry, the good effect of low level laser irradiation on increasing bone density and preservation of crestal bone around implants had been reported, El-Talawy et al., (2011); Rizk and El-din (2011); Ismaeel and Abbas (2011); El-Desouky et al., (2007). These studies showed that the alveolar bone height was preserved while the bone density was increased in the side exposed to low level laser and

depending on the good results obtained, the researchers concluded that application of laser to immediately loaded osseointegrated implants preserves the supporting alveolar bone and increases bone density compared to the unlased implants.

In the present study, the implants in one side were exposed to diode laser irradiation and the implants in the other side in the same patient were not exposed to any laser therapy, then both implants were early loaded and followed up for 6 months to evaluate the marginal bone around the implants. The results showed that the bone loss around the implants exposed to the diode laser was less than the marginal bone loss around the implants in the control side. The difference was significant at 3 and 6 months times. Regarding, the peri-implant probing depth, there was no significant difference between the two sides.

The results obtained in the present study is consistent with the results in other studies that showed that the bone around the implants could be preserved better, if area exposed to low level laser irradiation

than in the areas with no laser irradiation El-Talawy et al.,(2011); Rizk and El-din (2011); Ismaeel and Abbas (2011); El-Desouky et al.,(2007).

The early bone maturation and osseointegration after laser irradiation may be attributed to increase in number of viable osteocytes in the irradiated tissues that suggests that more vital bone tissue present in the irradiated area than in the non-irradiated area and that wound healing can be expected to be accelerated. In view of this data, low laser therapy appears to produce highly reactive and vital peri-implant bone tissue that can be expected that it could reduce healing times and speed up osseointegration of the implants, Dortbudak et al.,(2002). Kim et al.,(2007) suggested that application of the low level laser influenced the expression of certain cell activating factors and resulted in the expansion of metabolic bone activity and increased the activity of bone tissue cells.

**Conclusion:** Although, the number of the cases was few, we can conclude that the application of the diode laser to the implant can preserve the bone around it and may aid in improving the longevity of the implants.

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#### **5. References:**

- Colvard M, Kuo P. Managing aphthous ulcers: laser treatment applied. J Am Dent Assoc 1991;122:51-53.
- Dortbudak O, Haas R, Mailath-pokorny G. Effect of low power laser irradiation on bony implant sites. Clin Oral Imp Res 2002;13:288-292.
- Dortbudak O, Haas R, Mailath-Pokorny G. Biostimulation of bone marrow cells with a diode soft laser. Clinical Oral Implants Research 2000;11:540-545.
- El-Din MS, Yassin HH, Omar HM. Microscopic analysis and radiodensitometric evaluation of the effect of LLT around integrated dental implants under the influence of vitamin C: Experimental study. Egy Dent J 2008;54(3.2):20-49.
- El-Desouky G, Mekky M, El-Din MS, Zikry K, Amer W. Radiodensitometric assessment of the effect of diode laser on bone density following loading of lased endosseous implants. Egy Dent J 2007;53(2.2):12-23.
- El-Talawy DB, Michael C G, Mohie-El-Din HA, El-Din MS. Radiographic evaluation of low intensity irradiation on immediately loaded implant supporting overdenture. Egy Dent J 2011;57(3):2012-2018.
- Ismaeel SA, Abbas AH. The effect of low level laser on osseointegration of dental implants. J Bagh College Dent 2011;23(3):112-116.
- Kahraman SA. Low-level laser therapy in oral and maxillofacial surgery. Oral Maxillofac Surg Clin N Am 2004;16: 277-288.
- Khadra M, Ronold HJ, Lyngstadaas SP, Ellingsen JE, Haanaes HR. Low level laser therapy stimulates bone-implant interaction: An experimental study in rabbits. Clin Oral Implants Res 2004;15:325-332.
- Khadra M, Kasem N, Lyngstadaas SP, Haanaes HR, Mustafa K. Laser therapy accelerates initial attachment and subsequent behavior of human oral fibroblast cultured on titanium implant material. A scanning electron microscopic and histomorphometric analysis. Clin Oral Implants Res 2005;16:168-175.
- Kim YD, Kim SS, Hwang DS, Kim SG, Kwon YH, Shin SH, Kim UK, Kim JR, Chung IK. Effect of low level laser treatment after installation of dental titanium implant- immunohistochemical study of RANKL, RANK, OPG: An experimental study in rats. Lasers in Surgery and Medicine 2007;39:441-450.
- Lima AG, Antequera R, Peres MPSM, Snitcosky IML, Federico MHH, Villar RC. Efficacy of low- level laser therapy and aluminum hydroxide in patients with chemotherapy and radiotherapy-induced oral mucositis. Braz Dent J 2010;21:186-192.
- Luger EJ, Rochkind S, Wollman Y. Effect of low power laser irradiation on the mechanical properties of bone fracture healing in rats. Laser Surg Med 1998;22:97-102
- Mazzetto MO, Hotta TH, Pizzo RCA. Measurements of jaw movements and TMJ pain intensity in patients treated with GaAlAs laser. Braz Dent J 2010;21:356-360.
- Mester E, Spiry T, Szende B, Tota JG. Effect of laser rays on wound healing. Am J Surg 1971;122:532-535.
- Midamba E, Haanaes HR. Therapeutic effect of low level laser irradiation on inferior alveolar, mental and lingual nerve paresthesia. Laser Ther 1993;5:98-94.
- Petri Ad, Teixeira LN, Crippa GE, BelotibMM, Oliveira PT, Rosa AL. Effects of low -level laser therapy on human osteoblastic cells grown on titanium. Braz Dent J 2010;21(6):1-9.
- Rizk FN, El-din MS. Radiodensitometric evaluation of the effect of low energy laser irradiation with and without antioxidants therapy on immediately loaded implant supported overdenture. Egypt Dent J.2011;57(4):2977-2987.
- Saito S, Shimizu N. Stimulatory effects of low level laser irradiation on bone regeneration in midpalatal suture during expansion in rat. Am J Orthod Dentofacial Orthop 1997;111:525-532.
- Takeda Y. Irradiation effect of low-energy laser on alveolar bone after tooth extraction. Experimental study in rats. Int J Oral Maxillofac Surg 1988;17:388-391.
- Trelles MA, Mayayo E. Bone fracture consolidates faster with low power laser. Lasers in Surgery and medicine 1987;7:36-45.

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## The Impact of Regular Aerobic Exercise on the Levels of Leptin, Fasting Blood Glucose, Insulin and Insulin Resistance in Patients with Diabetes Mellitus Type 2

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**Abstract:** Diabetes mellitus is a metabolic disease, characterized by chronic increased blood glucose levels, cardiovascular diseases and disorders in carbohydrate, fat and protein metabolisms. Leptin, which represents the amount of fat tissue in the body, plays an important role in overall body metabolic regulation and glucose homeostasis. This study has aimed to determine the effect of 12 weeks' moderate aerobic exercise, on levels of leptin and glucose homeostasis factors, in patients with diabetes mellitus type 2. In this quasi-experimental study, 40 patients with diabetes type 2, who had been referred to the Parsian clinic, Mashhad, were divided equally into two groups, namely, an experiment group and a control group. The experiment group participated in a twelve-week program (3 sessions a week and on average 40 minutes per session, with 60 to 70 percent intensity of heart rate reserve) in aerobic exercises on Treadmill. In this study, the factors, including leptin, fasting blood glucose, insulin and insulin resistance (HOMA) were measured before and after the exercise-period. A significant difference was observed in the plasma levels of leptin ( $p = 0.000$ ), fasting blood glucose ( $p = 0.010$ ), insulin ( $p = 0.015$ ) and insulin resistance ( $p = 0.035$ ) in the aerobic exercises group compared to the control group. Leptin can play an important role in reducing the cardiovascular risks and improving the process of glucose metabolism in diabetic patients. The process is aided through regular aerobic exercises, which has the effect on the one hand of reducing insulin resistance and increasing glucose metabolism, and on the other hand, of reducing serum.

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**Keywords:** Aerobic exercises; Diabetes Type 2; Leptin; Glucose homeostasis

### 1. Introduction

The diabetes disease, sometimes known as the silent epidemic, is a disease in which the blood glucose levels are higher than normal. This disease is associated with increased urinary excretion and urinary glucose presence. Diabetes mellitus is a chronic metabolic disease that leads to damage to various organs of an infected person and reduction of longevity. According to the latest statistics, by the year 2025, the number of patients with this disease will reach to 135 million worldwide (1). In recent years, the prevalence and increase in obesity, diabetes and their related disorders are considered as the major health problems in Iran, and based on the same report, 58 percent of Iranian women and 75 percent of Iranian men suffer from overweight and obesity (2). In addition to this, 46 percent of mortality in Iran, caused by coronary vascular disorders, is due to hypokinesia, obesity and its complications - such as

diabetes (3). The basic process is one where blood lipid disorders and increased fat tissue associated with central accumulation pattern are observed in diabetic patients (4). Insulin resistance is considered as fundamental to the properties of metabolic syndrome and early disturbance in the development of diabetes type 2. Reports also suggest the role of insulin resistance in the development of cardiovascular disease (5). It is only so far that the impact of physical activity has been known in the treatment of diabetes mellitus (6). Researchers having considered different approaches in treating diabetes and its complications through physical activity and exercise. Due to the importance of glucose metabolism and its related factors, most research has focused on effective factors in regulating blood sugar.

Although most of the conducted studies have reviewed aerobic exercises' impact on this

disease, there are many differences between the results obtained (7). For example, a study has shown that 12 weeks of aerobic exercise has no effect on the factors in these patients, including their insulin sensitivity, fasting blood insulin and fasting blood glucose (8). Also, an accurate intensity of physical activity for favorable changes in lipids, blood pressure, glucose levels and insulin resistance has not been determined, so that both the World Health Organization and the American College of Sports Medicine have recommended exercise intensity ranges as 55 percent to 80 percent (9, 10). Produced in fat tissue, leptin is a hormone that has a circadian pulse secretion, its rate reaching a maximum during the night and decreasing nearing the dawn (11). After passing the blood brain barrier, the hormone has influence on the hypothalamus and causes reduced appetite and energy intake (12). Leptin has a direct correlation with volume and size of fat cells (13); on the other hand, it has a direct relationship with certain hormones such as insulin, so that an increase in leptin levels is associated with increased insulin resistance and diabetes type 2 (11). Houmard et al. (2000) have shown in their research that a short-term aerobic exercises course (60 minutes with the 75 percent  $VO_2$  max intensity in seven consecutive days) does not cause a change in leptin levels in healthy males, both in young and older groups (14). Regarding the same subject, Kraemer et al. (1999) have found no change in leptin levels in obese women after 9 weeks of aerobic exercises (15). Meanwhile Kumru et al. (2005) have concluded that both short-term and long-term aerobic exercises reduce leptin levels significantly, and that such a reduction has been in parallel with reduced body mass index (BMI) (16). Given the inconsistencies in the studies conducted, and the lack of research on simultaneous homeostasis factors of glucose and leptin, this study has aimed to investigate the effect of regular aerobic exercises on leptin levels and the levels of glucose homeostasis factors (insulin, glucose and insulin resistance) in patients with diabetes type 2.

## 2. Material and Methods

### 2.1. Statistical population and the subjects

The research design used in the study was a quasi-experimental method. The research statistical population consisted of all patients with diabetes type 2 who had been referred to the Parsian Diabetes Clinic in Mashhad. Among these individuals, those who volunteered were 40 people in the 35-45 year age range. Blood sugar levels ranging from 150 to 250 (mg/dl) were selected, using screening methods once interview and review of medical records had taken place. The subjects were then randomly divided into two equal groups that comprised an aerobic

exercises group and a control group. To avoid the influence of previous experience impacting on research results, only volunteers with no history of regular sports activity were selected. The conditions to be met in order to participate in the research included: female gender, diabetes type 2 both diagnosed by specialist physician and based on medical records, over 35 years old, and fasting blood glucose level ranging 150 -250 mg/dl. The exclusion criteria included: 1) currently suffering from other chronic diseases; 2) currently suffering from mental illness; 3) having maintained a regular exercise program over the past 3 months; 4) a history of myocardial infarction (heart attack); 5) uncontrolled arrhythmias; 6) third-degree heart block; 7) high blood pressure (over 100/200 in under treatment patients); 8) diabetes type 1; and 9) diabetic complications such as diabetic foot ulcers, nephropathy and microalbuminuria.

### 2.2. Exercise protocol

The group that was subject of the experiment participated in a twelve weeks program (3 sessions a week and on average 40 minutes per session, with 60 to 70 percent intensity of heart rate reserve) in aerobic exercises on Treadmill. The exercise intensity was controlled using polar heart rate monitor device (s625x). The control group was advised to avoid participating in any type of sports exercises during the 12 weeks of the experiment.

### 2.3. Nutrition control

A questionnaire was given out three days before blood sampling. Participants were asked to note the amount and type of food that they consumed. The purpose of this survey was to consider the effect of nutrition on insulin resistance and to control the feeding of the subjects. The researchers measured the calories of the people's diet, and designed and repeated a similar 3-day diet for secondary blood sampling.

### 2.4. Blood factors measurements

All subjects in both groups were referred to the laboratory at the same point in the day (8-10 a.m.), where the subjects had been fasting for 12 hours and had undertaken no physical activity 24 hours before the testing. In the laboratory, 20 ml. of blood was taken from each subject's elbow vein. The samplings were conducted similarly before and after the exercises. The leptin concentrations measurement was performed by radio-immunoassay (RIA) method, using a diagnostic Biochem kit made in Hungary. For measuring insulin levels, the same method was used – a Gama counter device together with the Immuno tech company IM3210 kit, made in the Czech Republic. The measurement of blood glucose levels was conducted through an auto analyzer biochemistry Selectra device, built by Mann Company that used

the enzymatic method. Finally, insulin resistance was evaluated through a homeostasis evaluation method (HOMA-IR) and was calculated according to following formula (17):

$$\text{HOMA-IR} = \text{fasting glucose [mmol / l]} \times \text{fasting insulin [\mu U / ml]} / 22.5$$

### 2.5. Measuring the body mass index (BMI)

The body composition analysis device, Tanita B\_C418 model, was used for measuring body mass index and weight of the subjects.

### 2.6. Statistical Analysis

Research data was processed with the help of SPSS software, version 18 (SPSS Inc. Chicago Illinois, United States). The central trend indices and dispersion indices were shown through descriptive statistics. The Kolmogorov-Smirnov test was used to review the data distribution types. To compare pre-test and post-test data means in each test group, the statistical correlated t test was used. All the statistical tests were performed at the 95 percent confidence level ( $p < 0.05$ ).

### 3. Results

Forty people in two groups of the aerobic exercise group (20 subjects) and control group (20 subjects) performed the research pattern up to the end of 12 weeks, and the data obtained from them were analyzed. Descriptive information related to the age, weight, height, BMI, current drug-dose drug and disease-period of the subjects is given in Table 1. Following the exercise program being performed, the experiment group showed a significant decrease in weight ( $p < 0.001$ ) and body mass index ( $p < 0.002$ ) (Table 1). Furthermore, plasma levels of leptin ( $p < 0.000$ ), insulin ( $p < 0.015$ ) and blood glucose ( $p < 0.010$ ), and, consequently, insulin resistance index ( $p < 0.035$ ) had decreased significantly in the experimental group -compared to the control group (Table 2).

**Table 1.** Profile of subjects in two experimental and control groups

Group	n	Age (years)	Diabetes history	Medicine treatment before participating in the research	Height (Cm)	Weight (kg)		Body mass index (Kg/m <sup>3</sup> )	
						B	A	B	A
Test	20	40.4±5.11	5.38±3.4	2 tablets (1 Metformin, 1 Glibenclamide)	164.5±5.5	B	78.7±7.84	B	29.3±3.45
						A	75.4±6.35	A	28.1±4.15
Control	20	38.3±2.45	5.2±2.4	3 tablets (1 Metformin, 2 Glibenclamide)	168.5±6.5	B	80.3±6.35	B	28.47±5.2
						A	81.8±7.2	A	29±2.3

\* Significance level ( $p < 0.05$ ) ; A: After; B:Before

**Table 2.** Comparison of leptin, glucose, insulin and insulin resistance in two experimental and control groups

Group	Control			Test		
	Pre-test	Post-test	P	Pre-test	Post-test	P
Leptin (ng/ml)	3.87±5.67	1.82±5.84	0.423	4.82±5.46	0.72±1.4	0.000*
Fasting glucose (mg/dl)	25.16±163.53	25.72±162.13	0.185	23.92±173	22.35±155.5	0.010*
Insulin (MU/ml)	6.74±11.89	11.18±13.62	0.367	4.98±13.85	2.01±10.04	0.015*
Insulin resistance (HOMA)	3.94±4.09	6.27±5.4	0.567	2.03±4.35	1.92±3.77	0.035*

\* Significance level ( $p < 0.05$ )

### 4. Discussions

Based on the research results, blood insulin levels in patients of the experiment group had been significantly reduced. The results of this research are consistent with the results from the studies of Bruce et al. (18), but inconsistent with the results of the studies of Cauza et al. (7), Segal et al. (8) Massi et al. (19) and Esfehiani et al. (20). The observed differences in outcomes could result from age and gender differences in the statistical samples and differences in intensity and duration of exercises in the different studies. Since blood insulin levels are

reduced during exercise, the base insulin level and glucose-stimulated insulin level will reduce (21). Exercise also leads to reduced mRNA levels, required to produce pro-insulin mRNA and glucokinase mRNA in the pancreas. It seems therefore that there are at least two cellular mechanisms to reduce insulin secretion. The first reason for this supposition is that pro-insulin indicates the reduction of insulin mRNA synthesis in the liver. The second is that since the glucokinase presence in the liver is essential for the pancreatic beta cells' sensitivity to glucokinase mRNA insulin,

the reduction in glucokinase levels may lead to reduced sensitivity of these cells to insulin, and will reduce the amount of its secretion (22).

The most important finding of the present research was the reduced levels of insulin resistance in healthy middle-aged men, as a result of 12 weeks of aerobic exercises. The effect of exercises on insulin resistance has been studied through a wide range of research - which has mainly reported improvement in insulin resistance as a result of doing exercises (23-26). Carrel et al. reported that physical activity over 9 months, based on school physical fitness movements, improved insulin sensitivity as well as the levels of inflammatory markers such as adiponectine and TNF- $\alpha$  (tumor necrosis factor - alpha) in normal-weight children (25). Rubin et al. also reported that intense levels of physical activity have a direct relation to insulin sensitivity (26). Selected athletes, running an average of 48 miles per week, had less insulin resistance and more insulin sensitivity, compared to healthy people with low physical activity (23). However, there are also some studies that report exercise to have no influence on insulin sensitivity improvement (24). In a research paper that reported an increase in physical activity of men attempting 300 steps per day (approximately 30 minutes' walking), there were no significant effects on the hs-CRP levels, as well as insulin resistance level. The researchers stated that the reason had probably been the low duration and low intensity of the exercises (24).

The research results also showed that fasting blood sugar levels in the experimental group had reduced significantly after the ending of the interventions. The current research results are consistent with the results of Massi et al. (19), Bruce et al. (18), Esfehni et al. (20), and Duncan et al. (27), but inconsistent with the results of Segal et al. (8) and Cauza et al. (7). The reasons for these differences can be due either to variation in intensity of exercise, or to their intermittent or continuous patterns of practice. Our results' differences with those of Cauza et al. could for example stem from our study sample including only diabetic men over 35 years old, while older men and women had participated in their study; perhaps they could not perform the exercises with an intensity similar to those subjects of the current research - due to aging. The studies show that muscle contraction has an insulin-like role, and sends a large amount of glucose into the cell, to be spent on energy production (28). The muscle contraction increases the membrane permeability to glucose, probably due to the increased number of glucose carriers in the plasma membrane (Glut4). When sports activities are undertaken, the amount of Glut4 in the exercised

muscles increases, which improves the insulin action on glucose metabolism (29).

One of the most important results of this study is the significant reduction ( $p = 0.000$ ) of leptin levels in the aerobic exercise group, compared to the control group. Considering the duration and intensity of performed exercises, the results of this study were consistent with many researchers, including Kumru et al. (2005) (16), Peeri et al. (30) and Oliveet al. (2001) (31). This would seem to imply that long-term exercise with moderate intensity leads to significantly decreased serum leptin concentrations. Despite the results obtained in this study, when Kraemer (2003) (32) reviewed the influence of intermittent exercises for progressive intensity effects on the leptin levels of individuals, no significant changes were observed. On the other hand, Zaccaria et al. (2002) (33) studied the effects of three endurance races on the leptin concentrations of 45 males, each of whom had participated in one of the three races. The results showed that only long-term endurance exercises, that required large energy consumption, were causing significantly reduced serum leptin levels, and no significant decrease was observed in half-marathon running and Nordic skiing.

In the present study, the reduced leptin levels can be attributed to the long-term (3 months) high caloric intake during exercises that were taken with the desired intensity. On the other hand, leptin represents the percentage of body fat and the balance of what is received and consumed as it is reduced in the body (34, 35). It has been observed in this study that, due to exercise, the body's mass index level and the weight of subjects in the test group, have been significantly reduced, and that this can be considered as one of the possible reasons for leptin reduction, as well as appropriate balance in internal body metabolism and caloric intake (36).

In summary, the research results showed that regular exercise can play an important role in the improvement of diabetes type 2, and also in the prevention of its development and complications. It can be inferred from the results that regular aerobic exercises may play an important role in reducing risks of cardiovascular diseases, and in improvement of the glucose metabolism in diabetic patients; this is due to reduced insulin resistance and increased glucose metabolism, as well as decreased leptin levels. Research into both the cell and the carriers of cellular glucose could be a good path by which to complete the findings of this research.

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#### References

- Azizi F, Salehi P, Etemadi A, Zahedi-Asl S. Prevalence of metabolic syndrome in an urban population: Tehran Lipid and Glucose Study. *Diabetes Res Clin Pract* 2003; 61:29–37.
- World Health Organization. Technical report series 894. Obesity, preventing and managing the global epidemic. Report of a WHO consultation. Geneva: World Health Organization; 2000.
- Chinikar M, Maddah M, Hoda S. Coronary artery disease in Iranian overweight women. *International journal of cardiology* 2006; 113:391-394.
- Susan S, Steven H, Michael H.D, Ralph B. D, Steven Sr.F, George K, et al. Relationship of abdominal visceral and subcutaneous adipose tissue with lipoprotein particle number and size in type 2 diabetes. *Diabetes* 2008; 57:2022-2027.
- Misra A, Alappan N, Vikram N, Goel K, Gupta N, Mittal K, et al. Effect of supervised progressive resistance exercise training protocol on insulin sensitivity, glycemia, lipids, and body composition in Asian Indians with type 2 diabetes. *Diabetes Care* 2008;31(7):1282.
- Anonymous. American Diabetes Association: clinical practice recommendations 1999. *Diabetes Care* 1999; 22(Suppl 1):S1-114.
- Cauza E, Hanusch-Enserer U, Strasser B, Ludvik B, Metz-Schimmerl S, Pacini G, et al. The relative benefits of endurance and strength training on the metabolic factors and muscle function of people with type 2 diabetes mellitus. *Arch Phys Med Rehabil* 2005;86:1527–33.
- Segal KA, Edano A, Abalos J, Albu J, Bland L, Tmoas MB et al. Effects of exercise training on insulin sensitivity and glucose metabolism in lean, obese and diabetic men. *J Appl Physiol* 1991;71:2402-11.
- American collage of sport medicine. The recommended quantity and quality of exercise for developing and maintaining fitness in healthy. *Med Sci Sport Exercise* 1990; 22:265-274.
- Hjeltnes N, Heijboer AC, Groot PC, Stal W, Birkeland K.. Effect of training intensity on physical capacity, lipid profile and insulin sensitivity in early rehabilitation of spinal cord injured individuals. *Spinal Cord* 2003; 41:673-9.
- Kanabrocki EL, Hermida RC, Wright M, Young RM, Bremner FW, Third JL, et al. Circadian variation of serum leptin in healthy and diabetic men. *Chronobiol Int* 2001; 18: 273–283.
- Heilbronn LK, Rood J, Janderova L, Albu JB, Kelley DE, Ravussin E, et al. Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. *J Clin Endocrinol Metab* 2004; 89: 1844–1848.
- Dengel DR, Pratley RE, Hagberg JM, Rogus EM, and Goldberg AP. Distinct effects of aerobic exercise training and weight loss on glucose homeostasis in obese sedentary men. *J Appl Physiol* 1996; 81: 318–325.
- Houmard JA, Cox JH, Maclean PS, Barakat HA. Effect of short-term exercise training on leptin and insulin action. *Metabolism* 2000; 49: 858–861.
- Kraemer RR, Kraemer GR, Acevedo EO, Hebert EP, Temple E, Bates M, et al. Effects of aerobic exercise on serum leptin levels in obese women. *Eur J Appl Physiol*;1999, 80:154–8.
- Kumru S, Ozmerdivenli R, Aydin S, Yasar A, Kilic N, Parmaksiz C et al. Effects of regular physical exercise on serum leptin and androgen concentrations in young women. *J Men's Health and Gender* 2005, Vol. 2, No. 2, pp. 218–222.
- Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R. Homeostasis model assessment: insulin resistance and  $\beta$ -cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28(7):412-9.
- Bruce CR, Kriketos AD, Cooney GJ, Hawley JA. Disassociation of muscle triglyceride content and insulin sensitivity after exercise training in patients with type II diabetes. *Diabetologia* 2004; 47(1): 23-30.
- Massi-Benedetti M, Herz M, Pfeiffer C. The effect of acute exercise on metabolic control in type II diabetic patients treated with glimepiride or glibenclamide. *Horm Metab Res* 1996;28:451-5.
- Esfahani M. Effect of physical training on blood glycemia, plasma insulin and cardiovascular risk factors in niddms. *Olympic games* 2007; 14(4 (serial 36)):17-24.
- Rowell LB, O'Leary DS, Kellogg DLJ. Integration of cardiovascular control systems in dynamic exercise. *Handbook of Physiology*,



- section 12, Exercise: Regulation and Integration of Multiple Systems. New York: Oxford University Press; 1996. pp. 770–838.
22. Izawa T, Komabayashi T, Mochizuki T, Suda K, Tsuboi M. Enhanced coupling of adenylate cyclase to lipolysis in permeabilized Adipocytes from trained rats. *J Appl Physiol* ,1991; 71(1): 23-9.
  23. Fontana L, Klein S, Holloszy J. Effects of long-term calorie restriction and endurance exercise on glucose tolerance, insulin action, and adipokine production. *Age* 2010;32(1):97-108.
  24. Gray S, Baker G, Wright A, Fitzsimons C, Mutrie N, Nimmo M. The effect of a 12 week walking intervention on markers of insulin resistance and systemic inflammation. *Preventive Med* 2009;48(1):39-44.
  25. Carrel A, Mcvean J, Clark R, Peterson S, Eickhoff J, Allen D. School-based exercise improves fitness, body composition, insulin sensitivity, and markers of inflammation in non-obese children. *J Pediatr Endocrinol Metab* 2009;22(5):409-15.
  26. Rubin D, McMurray R, Harrell J, Hackney A, Thorpe D, Haqq A. The association between insulin resistance and cytokines in adolescents: the role of weight status and exercise. *Metabolism* 2008;57(5):683-90.
  27. Duncan GE, Perri MG, Theriaque DW, Hutson AD, Eckel RH, Stacpoole PW. Exercise training, without weight loss, increase insulin sensitivity and postheparin plasma lipase activity in previously sedentary adults. *Diabetes Care* 2003;26(3):557-62.
  28. Cartee GO, Young DA, Sleeper MD, Zierath J, Wallberg-Henriksson H, Holloszy JO. Prolonged increase in insulin-stimulated glucose transport in muscle after exercise. *Am J Physiol Endocrinol Metab* 1999; 276: E494-9.
  29. Kern MJ, Wells A, Stephens JM, Elton CW, Friedman JE, Topscott EB, et al. Insulin responsiveness in skeletal muscle is determined by glucose transporter (Glut4) protein level. *Biochem J* 1990; 270(2): 397-400.
  30. Peeri M, Boghrabadi V, Amir khani Z, Hejazi S M. The Effects of Aerobic Training on Leptin, Cortisol and Serum Testosterone Levels In Obese and Lean Men. *Res on sport sci*,2009;22,99-116.
  31. Olive JL and Miller GD. Differential effects of maximal- and moderate-intensity runs on plasma leptin in healthy trained subjects. *Nutrition* 2001 ;17: 365–369.
  32. Kraemer RR, Aboudehen KS, Carruth AK, Durand RT, Acevedo EO, Hebert EP et al. Adiponectin responses to continuous and progressively intense intermittent exercise. *Med Sci Sports Exerc* 2003; 35: 1320–1325.
  33. Zaccaria M, Ermolao A, Roi GS, Englaro P, Tegon G, and Varnier M. Leptin reduction after endurance races differing in duration and energy expenditure. *Eur J Appl Physiol* 2002; 87: 108–111.
  34. Friedman JM and Halaas JL. Leptin and the regulation of body weight in mammals. *Nature* 1998; 395: 763–770.
  35. Havel PJ. Role of adipose tissue in body-weight regulation: mechanisms regulating leptin production and energy balance. *Proc Nutr Soc* 2000; 59: 359–371.
  36. Mendoza-Nunez VM, Garcia-Sanchez A, Sanchez-Rodriguez M, Galvan-Duarte RE, and Fonseca-Yerena ME. Overweight, waist circumference, age, gender, and insulin resistance as risk factors for hyper leptinemia. *Obes Res* 2002;10: 253–259.

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## Separation of surface and deep geological structures by application of band pass filter and statistical comparison with other methods

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**Abstract:** The observed gravity field's Fourier power spectrum is modeled with two equivalent-source layers, one for the shallower (residual field) geologic sources, and other one deeper (regional field) geologic sources. The depths and average physical property contrasts of the two of them are determined by fitting the observed gravity field's Fourier power spectrum Cordell (1985) with a two-layer spectral model. Each equivalent layer is simulated by a horizontal thin plate with spread randomly varying and distributed point density sources through it Cordell (1992). Adopting such a theoretical model for the Fourier power spectrum yields a stable and well-behaved filter transfer function. Like all band-pass filtering, the method is ineffective in the case of insufficient vertical separation between the shallow and deep geologic sources that gravity anomalies desired to separate. The key factor of this method is its iteration and repeatable, in dependent in result interpretation. More than a comparison between this method with polynomial one was conducted and the correlation coefficient was determined. The results of this study showed that increase of polynomial degree correlation has a straight relationship with Wiener filter one.

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**Key word:** Wiener band-pass filter, regional field, Fourier power spectrum

### Introduction

The primary goal of studying detailed gravity data is to provide a better understanding of the subsurface geology Jacobsen (1987). The gravity method is a relatively cheap, non-invasive, non-destructive remote sensing method that has already been tested on the lunar surface.

Measurements of gravity provide information about densities of rocks underground. There is a wide range in density among rock types, and therefore geologists can make inferences about the distribution of strata. In the Taos Valley, we are attempting to map subsurface faults. Because faults commonly juxtapose rocks of differing densities, the gravity method is an excellent exploration choice Clark (1984).

Several methods such as second derivative for gravity separation methods are used for separation of other fields such as different geological structures such as layered and vertical structures.

Clark (1969) showed that the presence of noise can lead to divergences for downward continuation and differential maps and then Cordell (1985) modeled two layer sources by designing filter. The aim of this work is to design a band pass filter between different depths depending on the area and separation of local field from regional field, which is explained by Wiener (1949). The filter design process is simple and easily automated, being well-suited for modular implementation in a 'filter toolkit' applications program running on either a workstation or personal computer and yields repeatable, interpreter-independent results.

This study is an attempt to implement the new approach that can be stated in a separate layer structures.

### Filter defining in layers

Gravity anomaly detection filter problem addressed by the gravitational field when the anomaly is created by the separation of power spectral Fourier gravity observations are obtained.

Get rid of unwanted frequencies, highlight signals of certain frequencies, identify harmonic signals in the data, correcting for phase or amplitude characteristics of instruments, prepare for down-sampling and avoid aliasing effects are the main reasons for filtering Gupta (1980). The power spectrum in the frequency of long distance and short-distance frequencies is done by increasing the depth of the source.

Observed gravity field,  $g$ , (gravitational vertical component) is often described as a combination of deep and shallow geologic parameters (Pawlowski, 1993) as:

$$g_o = g_s + g_d \quad (1)$$

The gravitational field is defined by the sum of observed regional and residual gravity fields and Fourier power spectrum for two layers with different depths and average physical properties can be modeled, which the second layer belongs to deep sources.

Each layer is a thin horizontal density distribution of random sources which are considered to be transient. The average spectrum of the gravitational potential for a point source in a layer of depth,  $h$ , followed by Naidu, (1968) is modeled as follows:

$$\langle |G(f)|^2 \rangle = c \langle |\sigma|^2 \rangle \exp(-4\pi fh) \quad (2)$$

Where:

- ✓  $G(f)$  is the mean radial gravitational field Fourier of layer as a function of radial frequency ( $f$ ).
- ✓  $c$ , is a constant and  $\sigma$  is the constant density of point source (Cordell, 1979).

It is important to note that modeling of layer density distribution on base of random distribution of point sources is constant.

From equations (1) and (2) the mean radial Fourier spectrum the observed gravity field is modeled as follows:

$$|G_o(f)^2| \propto \langle |G_o(f)^2| \rangle = c \left[ \langle |\sigma_d^2| \rangle \exp(-4\pi fh_d) + 2 \langle |\sigma_d^2| |\sigma_s^2| \cos(\theta_d[f] - \theta_s[f]) \rangle \times \exp(-2\pi fh_d) \times \exp(-2\pi fh_s) + \langle |\sigma_s^2| \rangle \exp(-4\pi fh_s) \right] \quad (3)$$

Detection of local gravity filtered through the modeling was done between 5 to 25 km depth, be in the best fit with the observed values. To determine the resolution of selected method in the present work, the following simplified algorithm was performed (Cordell, 1991).

1. Conducted a guess for ( $h_d$ ) and ( $h_s$ ) to test in the studied area between depths of 5 to 25 km from the surface of the earth.
2. By increasing the  $h_s$  and  $h_d$ , can find a proven straight relation between  $h_d$ , with depths increasing, and can resolve the equation by putting  $\langle |\sigma_s| \rangle$  and  $\langle |\sigma_d| \rangle$ .

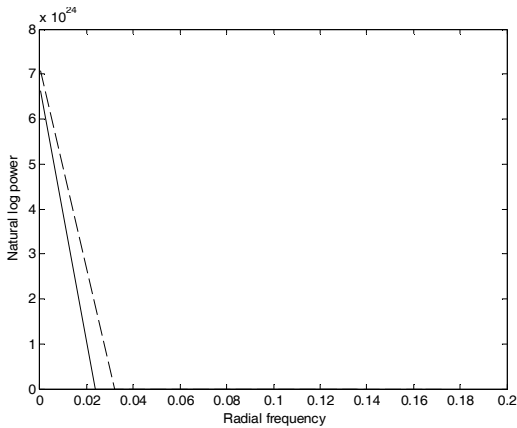
3. Repetitions the previous step while the  $h_d$  cover the full range (25 km).
4. Increasing the  $h_s$  for any 5 km in each calculation.
5. Repeat steps 2 to 4 to cover all of the area.

According to mentioned steps, figures 1 and 2 shows the designing band pass filter and radial fitted average power spectrum of observed gravity. These figures are indicating the two source layers modeled and drawing by regional field.

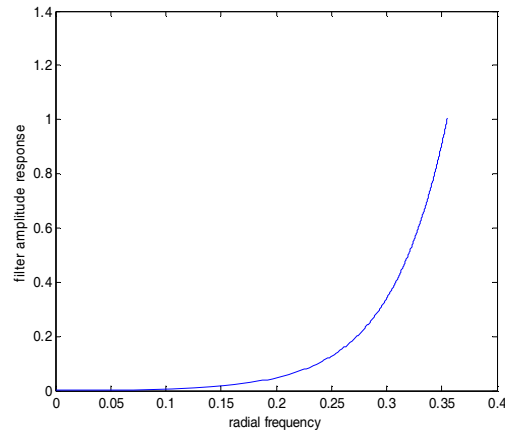
By application of the second equation, the coefficients are determined for of the first and second degree polynomials are shown in figure3 and 4.

$$g_R = a_0 + a_1x + a_2y + a_3 \Rightarrow g_R = 0.001 + 0.0007x + 0.0041y + 1.9xy \quad (4)$$

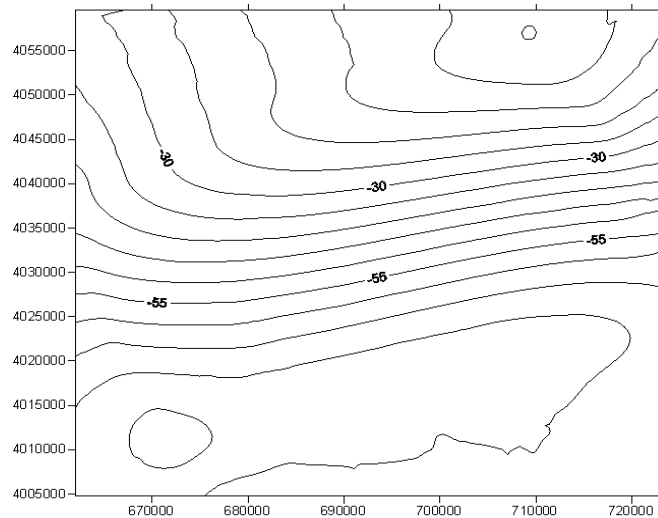
$$g_R = b_0 + b_1x + b_2y + b_3xy + b_4x^2 + b_5y^2 + b_6x^2 \Rightarrow g_R = 0.125x - 0.0918y + 0.0793x^2 \quad (5)$$



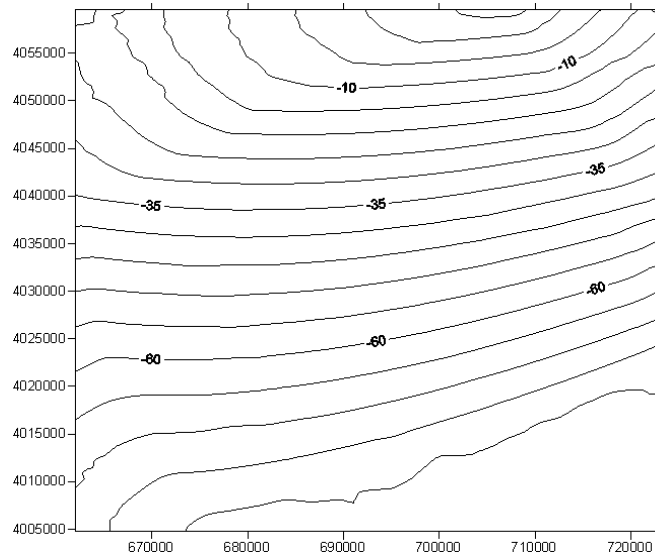
**Figure1.** Average radial power spectrum to estimate the depth



**Figure2.** Regional field based on band-pass Wiener filter design



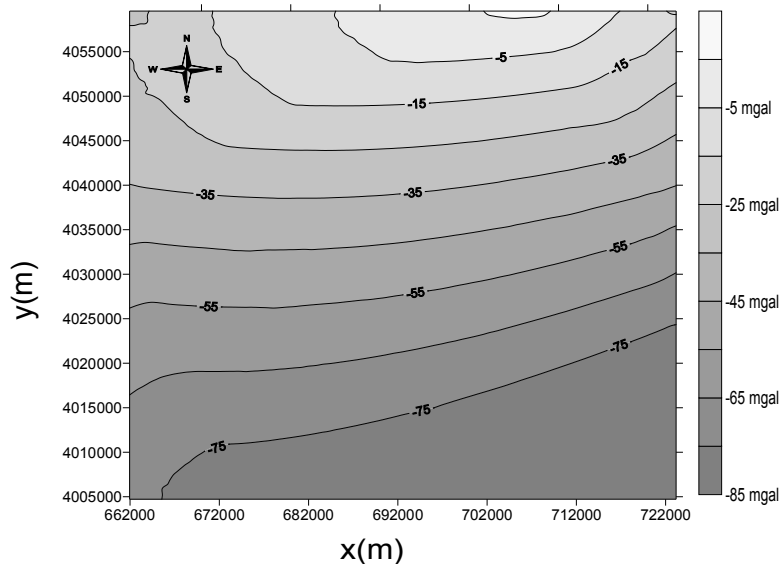
**Figure3.** Regional Median degree polynomial fitting method (first degree), with 5mgal contour lines



**Figure4.** Regional Median degree polynomial fitting method (second degree), with 5mgal contour lines

By defining the Band pass wiener as below, the figure5 can be obtained (Craig, 1996).

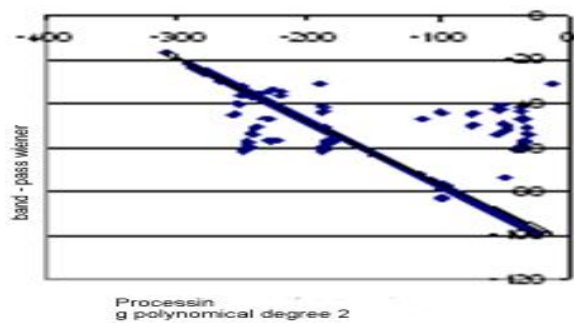
$$\tilde{H}(f) = \frac{1}{1 + \frac{G_s(f)}{G_d(f)}} \tag{6}$$



**Figure5.** Regional Median causeway Wiener filter method, with 10mgal contour lines

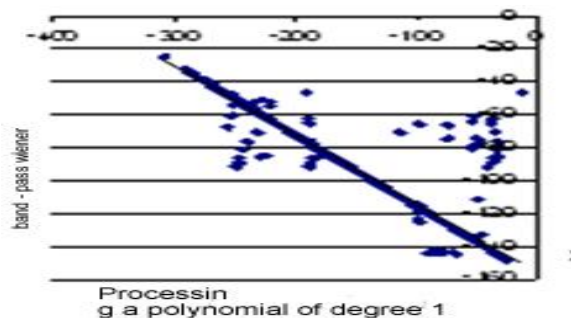
**Conclusion:**

In this thesis paper, Wiener filtering method can be used as a method to isolate the local fields and the remainder was introduced to evaluate the performance of the analytical



**Graph2.** Band pass Wiener filtering method and the correlation coefficient fit for second degree polynomials

method with polynomial fitting and compared both that Causeway Wiener filtering method and the square of the Pearson fitting a quadratic polynomial 1 is  $R^2 = 0.9475$ . Pearson Square causeway Wiener filtering method and the polynomial fit of degree 2,  $R^2 = 0.9491$ , respectively.



**Graph1.** Band pass Wiener filtering method and the correlation coefficient fit for first degree polynomials

**Reference**

- Clarke, G. K. C., 1969, Optimum second-derivative and downward continuation filters, *Geophysics*, 34, 424-437.
- Clark, R. D., 1984, Lucien LaCoste, *The Leading Edge*, 3, 24-29.
- Cordell, L., 1979, Gravimetric expression of graben faulting in Santa Fe county and the Espanola Basin, New Mexico, in Ingersoll, R.V., ed., *Guidebook to Santa Fe County, 30th Field Conference*, 59-64, New Mexico Geological Society.
- Cordell, L., 1985, A stripping filter for potential-field data, *55th Ann Internal* 218.
- Cordell, L., 1992, A scattered equivalent-source method for interpolation and gridding of potential-field data in three dimensions: *Geophysics*, 57, 629-636.
- Cordell, L., Zorin, Y.A., and Keller, G.R., 1991, The decompensative gravity anomaly and deep structure of the region of the Rio Grande rift: *Journal of Geophysical Research*, 96, 6557-6568.
- Gupta, V. K., and Ramani, N., 1980, Some aspects of regional residual separation of gravity anomalies in a Precambrian terrain *Geophysics*, 45, 1412-1426.
- Jacobsen, B. H., 1987, A case for upward continuation as a standard separation filter for potential-field maps: *Geophysics*, 52, 1138-1148.
- Craig, M., 1996, Analytic signals for multivariate data, *Mathematical Geology*, 28, no. 3, 315-329.
- Naidu, P., 1968, Spectrum of the potential field due to randomly distributed sources: *Geophysics*, 33, 337-345.
- Pawlowski, R. S., and Hansen, R. O., 1993, Gravity anomaly separation by Wiener filtering, *Geophysics*, 55, 539-548.
- Wiener, N., 1949, *Extrapolation, interpolation, and smoothing of stationary time series*, John Wiley and Sons, Inc.

11/10/2012

**Studying of performance of the under construction drainage system in shiraz plain****Ahmad Reza Karimipour<sup>1</sup>, Golnoosh Banitaleby<sup>2</sup>, Iman karimipour<sup>3</sup>, Mehdi Ahmadi<sup>1</sup>**1. Instructor of civil and hydraulic structure, Ardal training center, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran (corresponding Author) [Ahmadrezakarimipour@yahoo.com](mailto:Ahmadrezakarimipour@yahoo.com)1. Instructor, Shahrekord Branch (Ardal center), Islamic Azad University, Department of Mechanic Engineering, [mehdiahmadi81@gmail.com](mailto:mehdiahmadi81@gmail.com)2. M.Sc. Student of Soil physics and conservation, Agriculture Faculty, Shahrekord University, Iran, [golnoosh.banitalebi@gmail.com](mailto:golnoosh.banitalebi@gmail.com)3. M.Sc. Student of Solid Mechanics, Mechanical Engineering Department, Faculty of Engineering, Yazd University, Iran, [iman.karimipour@gmail.com](mailto:iman.karimipour@gmail.com)

**Abstract:** Shiraz plain, covering an area of roughly 300 km<sup>2</sup> and having an average altitude of 1500 meters, is located in Fars province, in the South of Iran, in a climatologically arid and semi-arid region. Due to the problems emanated from elevated water level in parts of Shiraz plain, some drainage system constructions have been implemented with different purposes, the most significant of which are drawing down the water table in South East Shiraz and transferring water to Sarvestan plain. These projects were studied and initiated by Fars Regional Water Organization in 1993 and they are at operational stages nowadays. In the present study, after investigating the factors affecting elevated groundwater level in Shiraz plain and Shiraz city and examining such prevention techniques as the use of a drainage system, the effects of the aforementioned projects on averting water table rise in Shiraz plain in the future were simulated via PMWIN Model. After calibration and validation of the model, the required parameters were determined and groundwater level in the plain with and without the drainage system was simulated for four different cases. The results of all cases indicated that although lowering the elevated groundwater level project at South East of the plain and Shiraz urban sewage collection system were both being carried out simultaneously, in most parts of the study area, groundwater levels did not go down to the expected extent (10 meters), and hence, Khatoon drainage alone cannot solve the elevated water table problem. There is, accordingly, a need for more drainage lines in the plain.

[A. R. Karimipour, G. Banitaleby, I. Karimipour, M. Ahmadi. **Studying of performance of the under construction drainage system in shiraz plain.** *Life Sci J* 2012;9(3):954-966]. (ISSN: 1097-8135).<http://www.lifesciencesite.com>. 137**Keywords:** Ground water, Shiraz plain, Drainage system, PMWIN, Modeling, Effective parameters**1.Introduction**

In general, ground water resources do play a significant role in meeting the water demands in an area. Thus their evaluation, simulation, and management are vitally important. Shiraz plain, in particular, is facing elevated groundwater level problem in its southeastern region due to phenomena like increasing population, conversion of farmlands and gardens into residential areas, and destruction of old aqueducts that used to drain the plain. To overcome this problem, construction of three drainage aqueducts in the plain has been underway since 2003. So far, more than half of one of these aqueducts, with a rough length of 15 km, has been constructed. Examining the effectiveness of this drainage system, and predicting its function in the future necessitate more research in this area.

Many studies in the field of ground water flow simulation have been conducted. The following sketch makes a mention of just a few of these studies: the study of simulating groundwater flow in multi-aquifer systems with analytical and numerical Dupuit models by Bakker (1999), modeling ground and surface water interactions using Dupuit approximation by Anderson (2005), and the reconstruction of ground water parameters from head in an unconfined aquifer by Yan et al, (2007). Finite

difference method for simulation of different aquifers has also been employed by different researchers. Projects such as numerical modeling of ground water resource management options in Kuwait by Mukhopadhyay et al. (1994), development and application of a comprehensive simulation model to evaluate impacts of watershed structures and irrigation water use on stream flow and ground water by Ramireddygar(2000) and studying Bajgah plain ground water situation using the finite difference three dimensional modular MODFLOW model by Rezaei and Mousavi are among the examples of such projects [4-6].

The application of MODFLOW model as a modular three dimensional finite difference model to predict behavior groundwater has undergone noticeable developments during in more recent years. Such studies as using sensitivity analysis to assist parameter zonation in groundwater flow model by Jiao (1996), modeling stream aquifer seepage in an alluvial aquifer by Osman et al. (2002), and modeling water balance in Rio Turbio aquifer, Mexico by Johannes (2004) are instances of these research projects [7-9]. Other research studies that have been conducted in this field are for example MODFLOW equipped with a new method for the accurate

simulation of axisymmetric flow by Samani et al. (2004), fully conservative coupling of HEC – RAS with MODFLOW to simulate stream – aquifer interactions in a drainage basin by Rodriguez et al. (2008), and a comparison of groundwater fluxes computed with MODFLOW and a mixing model using Deuterium: Application to the Eastern Nevada test site and vicinity by Rosemary et al. (2008).

In the present study, however, the use of drainage system for dropping groundwater table of the Shiraz plain has been evaluated. For this purpose, at first Shiraz plain aquifer hydraulic behavior was modeled using PMWIN model, the core of which is formed on the basis of MODFLOW software. In this model, the performance of a recently constructed drainage system in the plain was modeled and parameters affecting hydraulic behavior of the aquifer were analyzed. Measured rainfall and evaporation rates in the plain, water recharge and discharge rates through the aqueducts and the Khoshk and Chenarrahdar Rivers, the amount of water discharged from water wells, as well as recharge rate due to returned wastewater were all considered in the model. Plain hydrodynamic coefficients were estimated by calibrating the model, and sensitivity analysis of the model was performed for four important parameters. In the end, groundwater level in the plain with and without the drainage system was simulated for four different cases.

## 2. Introducing Shiraz Plain

Shiraz plain is stretched from north to Babakoohi and Kaftarak mountains, from northwest to Derak mountain, from south to Sabzpooshan and Soltanabad mountains and from west and southwest to Polfasa mountain and

Maharloo lake. The area of this plain is roughly 300 Km<sup>2</sup> and its location is shown in Figure 1. Studies have shown that the Shiraz alluvium plain is layered, and clay layers are located between the aquifers. The alluvial sedimentation does not have a uniform thickness and sandy layers are located between silt and clay layers. Also Geophysical explorations indicate that Shiraz plain aquifer goes down as far as 200 m deep, and at depth below that if there is an aquifer layer at all, it does not have a good quality [13]. Furthermore, the alluvium structure in the west plain is mainly coarse grain and it turns to fine grain near Maharloo lake.

Based on these studies, Shiraz plain groundwater is divided into two aquifers, namely surface groundwater and deep groundwater. Surface groundwater goes down to a water table to the depth of 40 m, while deep groundwater is ranges from about 40 m of depth to 200 m.

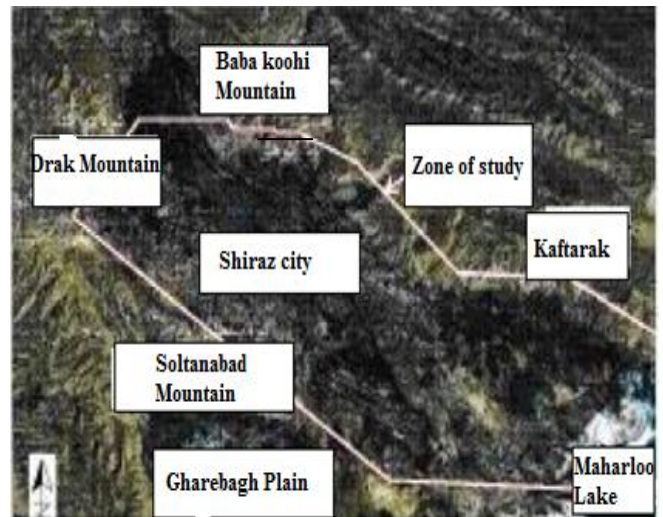


Figure 1. Shiraz Plain and the Area of study

In the range of the study area, there are 35 active observation wells and 35 active piezometric wells. During the implementation of Khatoon drainage, 12 piezometric wells surrounding the drain have been created for reading water levels. In this study, monthly fluctuation statistics of water tables of 41 rings of wells were collected and used. There are also a number of 425 rings of operating wells of surface groundwater in zone of the study that are mostly used for agricultural purposes. In Table 1 the seasonal operating of flow from two rings of wells is shown.

Table 1. Characteristics of Two Operating Wells in the Shiraz Plain

UTM (x)	UTM (y)	Spring Discharge (m <sup>3</sup> /d)	Summer Discharge (m <sup>3</sup> /d)	Fall Discharge (m <sup>3</sup> /d)	Winter Discharge (m <sup>3</sup> /d)
666901	3273497	-217	-379	-392	-112
657624	3273984	-346	-412	-398	-170.4

## 3. Simulation of Shiraz Plain Aquifer and Appropriation of Parameters to the Model

Shiraz plain aquifer network consists of 15500 cells and contains 100 rows and 155 columns and each cell is divided into the dimensions of 200\*200 meters. Active cells in the model were symbolized with 1 and inactive cells with 0 and fixed-head cells with -1. The study area is mostly surrounded by elevations; hence, only 32 cells from the western boundary and 63 cells from the southeast boundary were at hydraulic exchange with areas outside the study area. In terms of general eastward groundwater flow, the active cells located in the western boundary are the

cells receiving groundwater flow from outside of boundary, and are called GHB cells in the model. Active cells located in the southeastern boundary are the cells discharging outflow outside the zone and are called discharge cells. Illustration of gridding of Shiraz plain aquifer and grid cells are shown in Figure 2.

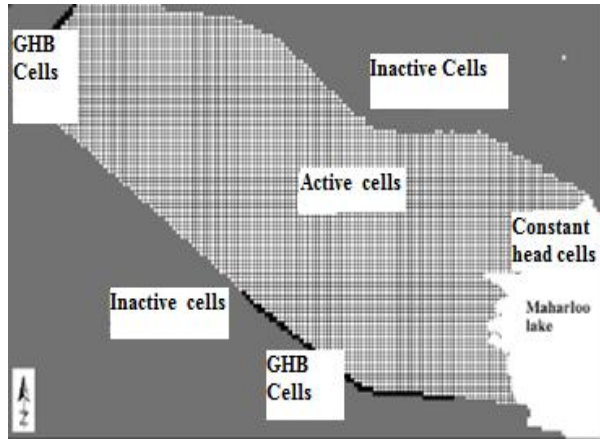


Figure 2. Gridding System of Shiraz Plain Aquifer and Its Grid Cells

The modeling of Shiraz plain is performed (in the case of transient) within a three-month period. To solve the differential equation, each time period was divided into three one-month-period phases. Total simulation period lasted more than 42 months (from March 2005 to November 2008). The data obtained from the first 30 months were used for the model calibration and for determination of model hydrodynamic coefficients, while the data from the following 400 days were used for validation and model sensitivity analysis.

To determine the initial values of specific storage and hydraulic conductance, the measurements carried out on the hydraulic conductivity and specific yield in 11 different wells in the plain area were used. The results of their distribution in the plain are shown in Figure 3 and Figure 4, respectively.

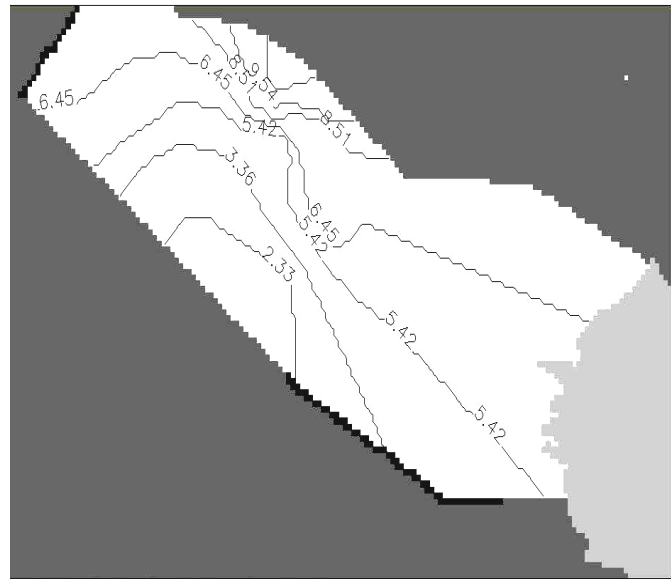


Figure 3. Distribution of the Initial Values of Hydraulic Conductivity (m/d) in Different Zones of the Plain

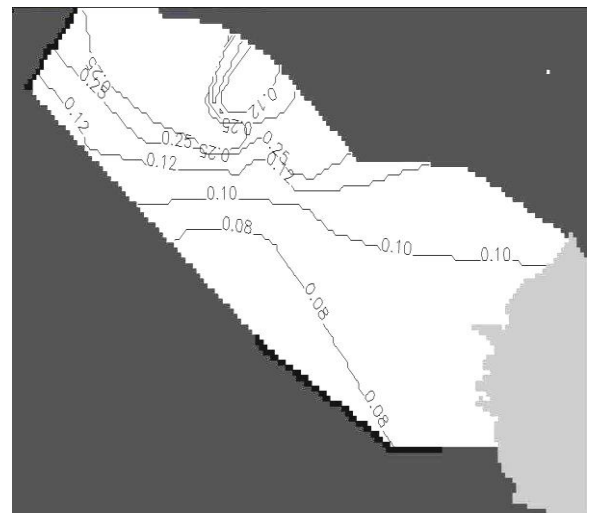


Figure 4. Distribution of the Initial Values of Specific Yield in Different Zones of the Plain

The level of the impervious layer of the bottom of the aquifer was obtained from existing maps, and through interpolation, the figures for that layer were obtained at 200 m distances in the whole plain (Figure 5). The same procedure was exercised for the data obtained from the elevation points of the plain and the topographic map of the plain at 200 m distances was prepared (Figure 6).



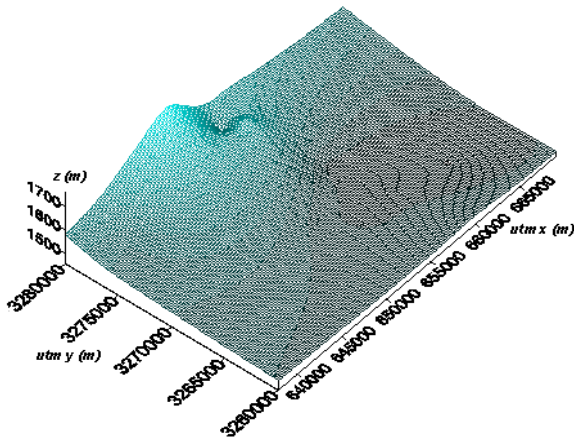


Figure 5. The Level of the Impervious Layer of the Bottom of the Aquifer

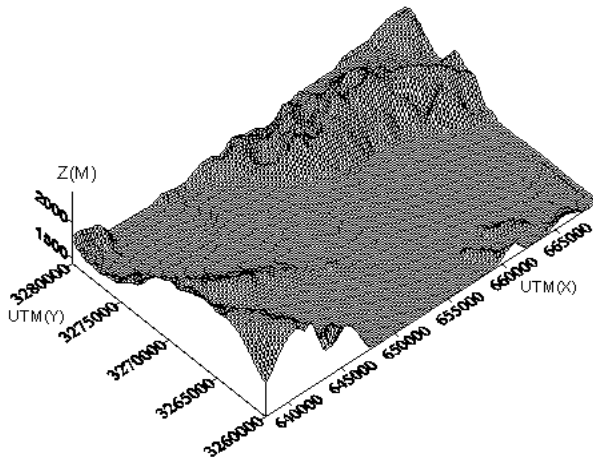


Figure 6. Topographic Map of the Shiraz Plain.

By subtracting the values of the impervious layer level of the bottom of the aquifer from the ground level, the thickness cells in the model was determined and then imported into the software. To determine the evapotranspiration of the model, the statistical data from Shiraz airport evaporation station were used.

**3.1- Recharge Resources**

One of the groundwater recharge resources is infiltration of precipitation. To calculate the infiltration result of atmospheric precipitations at each time step, the values of primary losses and runoff were reduced from the monthly precipitation average. According to the previous studies, the first 1.6 mm of the precipitation was subtracted for reasons of initial losses, and then to determine the amount of runoff, the division of the plain in terms of population of different zones (Figure 7) and runoff coefficient for each region were used (Table 2).

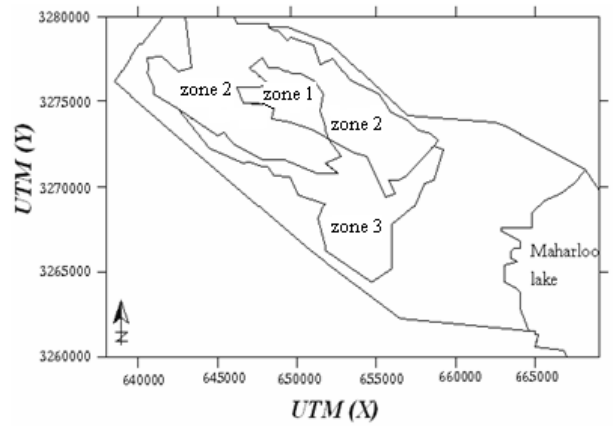


Figure 7. Division of the Plain in Terms of Three Population Zones

Table 2. Runoff Coefficient of the Triple Zones

	Triple zones	Run off coefficient
1	compressed	0.65-0.75
2	average density	0.5-0.6
3	Few density	0.35-0.4

Another groundwater recharge resources is the return of backwater to the groundwater. According to the research and prediction done by the Housing and Urbanization Department of Fars Province, the amount of monthly water consumption and monthly produced waste water at each region of the plain was proven to be attributed to the population density in that area. Therefore, using the population density classification (Figure 7) and wastewater per capita production, the amount of produced wastewater in each time step was calculated. Table 3 depicts the amounts of minimum, average and maximum consumption of water per capita and waste water per capita production from 2001 to 2011.

Table 3. The Amounts of Consumption of Water Per Capita and Wastewater Per Capita Production from 2001 to 2011 in Shiraz [14].

Year	2001			2011		
	minimum	average	maximum	minimum	average	maximum
population	1738000			2488000		
description	minimum	average	maximum	minimum	average	maximum
consumption of water of per capita(lit/day)	143	190	257	149	198	267
wastewater per capita production(lit/day)	110	147	198	122	163	220

The amount of agricultural return water to the ground water, as a percentage of the total amount of

irrigation water, can usually be produced and it will be different in different months of the year. According to the past studies, [13] the monthly volume of input water to the surface ground water, due to the harvest of agricultural water from the deep ground water, is shown in the Table 4.

Table 4. The Volume of Input Water to the Surface groundwater due to the Harvest of Agricultural Water from the Deep Groundwater [15].

Time period	spring	summer	autumn	winter
volume input water (1000 M <sup>3</sup> )	3019	3019	3019	0

### 3.2. Discharge Resources

One of the important sources of aquifers discharge is the water harvest of wells. To calculate the rate of water harvest, statistical data were gathered from all the wells, and the wells' depth, type of use, discharge and the number of hours of pumping in a day have been figured out [13].

Some amount of water harvested in different ways will be returned to the groundwater. The amount of water back to the groundwater is calculated and finally the amount that is actually taken out of the groundwater throughout the year and is effective in reducing the level of water table is entered into the software as GRD matrix file (due to the high multiplicity of date). The situation of these wells is shown in Figure 8.

This contains shallow wells operating in the whole region (425 wells) and the temporary pumping wells around the drainage path that are used for drying drainage drilling path (500 wells). After passing the drainage from the temporary wells, these wells become inactive with some of them only in function four hours a day.

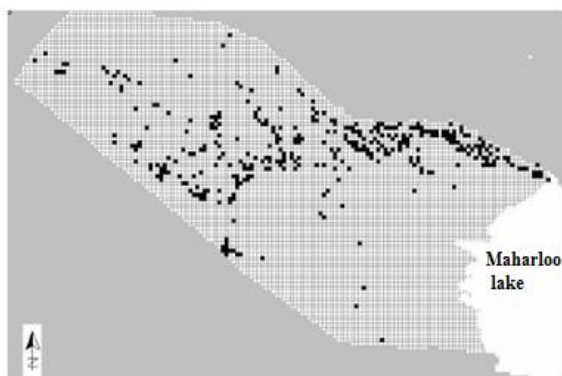


Figure 8. Exploitation Wells and the Temporary Pumping Wells around the Drainage in Shiraz Plain.

Drainage network constructed is another source of aquifer depletion. After networking the aquifer,

Khatoon drainage constructed path was located in 76 cells. Figure 9 shows the situation of Khatoon drainage and the ambient rivers.

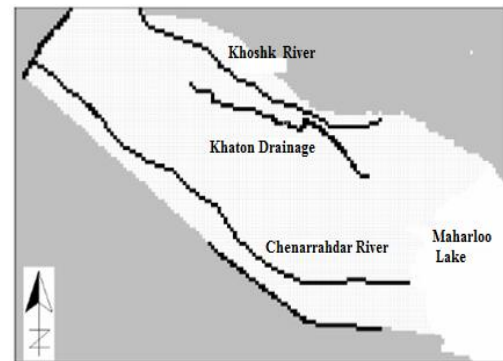


Figure 9. Khatoon Drainage and the Ambient Rivers in the Model

To calculate the flow entered into the drain for each of cells, the equations 1 and 2 were used which are similar Darcy's Law, and their parameters were calculated for all 76 cells.

$$Q_D = C_D (h/d)^2 \quad h > d \quad (1)$$

$$Q_D = 0 \quad h < d \quad (2)$$

Here CD is Drain hydraulic conductance and it depends on the material characteristics of the drain and environmental conditions and (h) is the hydraulic head in a drain-cell. D is the elevation of the drain. Equation 2 ensures that in case the discharge to the drain will be zero when the hydraulic head is lower than or equal to the drain elevation.

And the amount of hydraulic head for each cell,  $h_{i,j,k}$  in each time step was determined by the software. Based on current measurements [16] the value of 20 m<sup>2</sup>/day was considered as the initial estimation for the coefficient of CD and based on that, the simulation was conducted and then this coefficient was optimized in the calibration stage.

### 3.3- Determining Initial Conditions

The initial conditions are the most important parameters for solving partial differential equations in the ground water and the calculations should begin with these condition considered. Because the model simulation started in 2005, statistical data from the water table since march 2005, as the initial transient conditions, was given to the model. Figure 10 shown the initial water table level of march 2005 in the model cells. As expected, the water table shows the general flow of direction from the west to east in the plain.

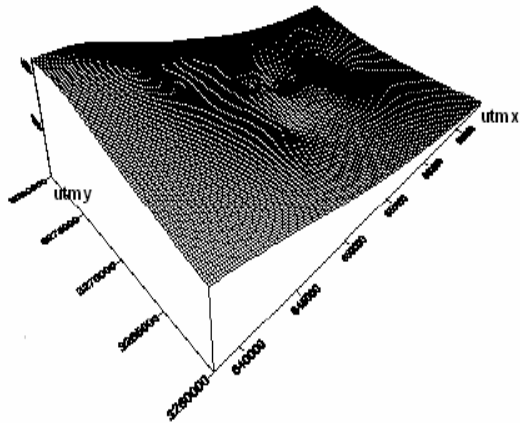


Figure 10. Initial Water Table Level in March 2005

**4. Model Calibration**

The result of the first implementation of the model (before calibration) as the calculated water table level was compared with observatory water table level in nine wells and is shown in Figure 11.

As is clear, the calculated values in most wells are higher than the observed values and the variance obtained is higher than 19 m<sup>2</sup>.

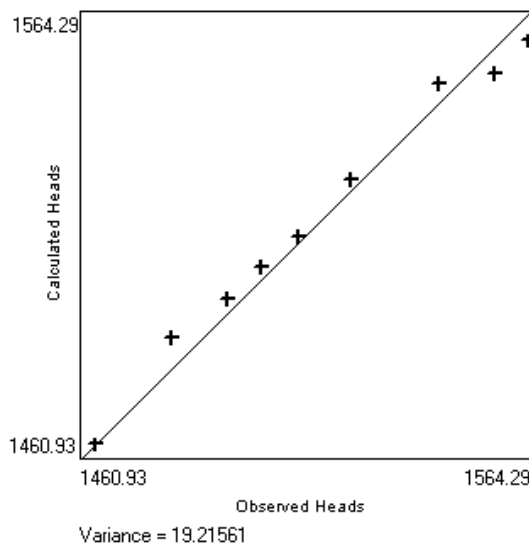


Figure 11. The Comparison of Calculated Water Table Level Values and the Observed Values before Calibration.

Since the aquifer hydrodynamic coefficients had different values (due to the geological context of the region and the heterogeneous aquifer in the different parts of the plain), the area under focus was divided into several smaller areas on the basis of the texture in order to determine the hydraulic conductivity coefficient and specific yield. With implementation of sequential model for the different amounts of these coefficients, the model was calibrated in such a way

that the best correspondence between observed water table level and the calculated water table levels in the wells could be obtained.

Figures 12 and 13 respectively illustrate the hydraulic conductivity and specific yield zoning after model calibration.

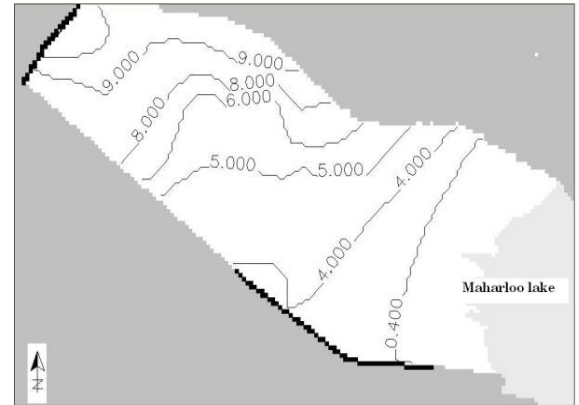


Figure 12. Hydraulic Conductivity of Zoning in the Plain after Calibration.

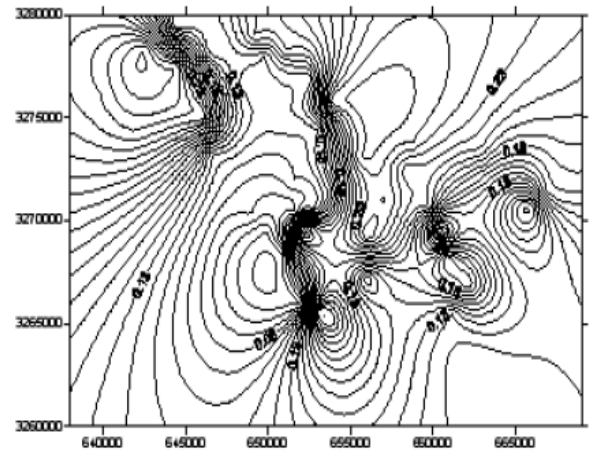


Figure 13. Specific Yield of Zoning Diagram after Calibrating the Model.

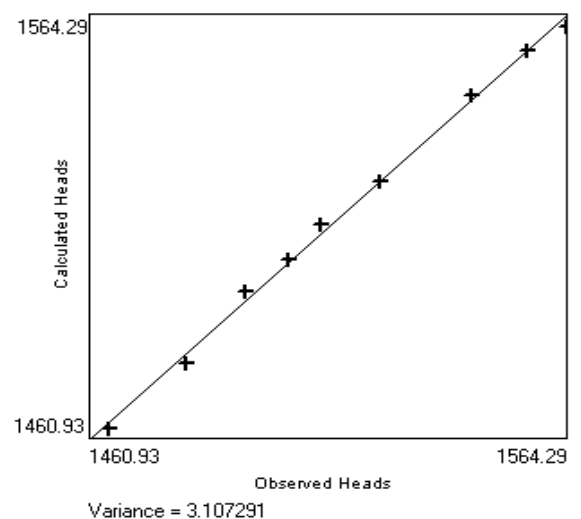
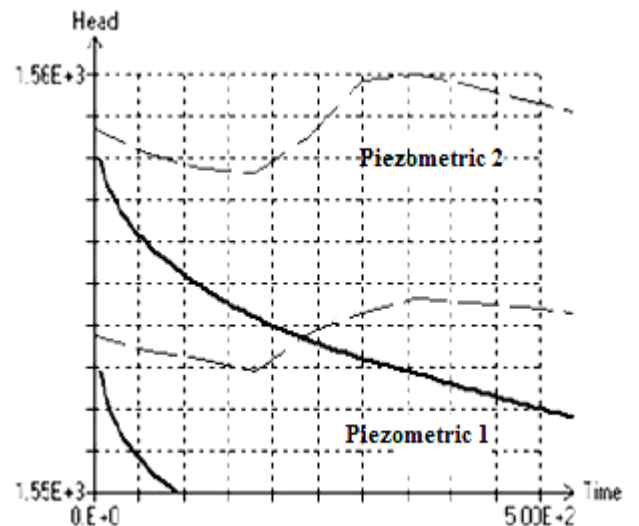


Figure 14. The Scatter Diagram after Calibration.

As the comparison of Figures 11 and 14 implies, the implementation calibration causes a correspondence between measurement and simulation, such that the Variance has reduced about  $3m^2$ .

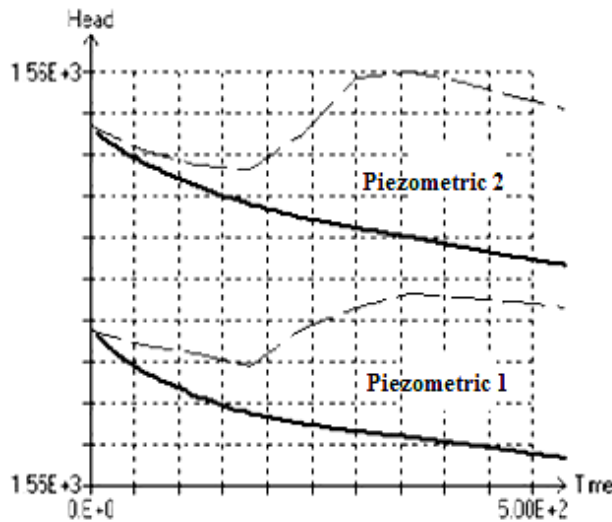
**5. Sensitivity Analysis Results**

Model sensitivity analysis was performed for several parameters. For reasons of space, very briefly the effect of only four important parameters is dealt with. Aquifer Recharge resources was the first parameter that was analyzed. It was revealed that: firstly, the amount of recharge can be estimated low or high very easily. Secondly, the model is so sensitive to recharge rate that a ten-percent increase or decrease in recharge can change the water table level in some parts of the plain as deep as 7 meters. Figure 15 shows changes in calculated water table level in piezometric wells 1 and 2, caused by 10 percent increase or decrease in recharge rate.

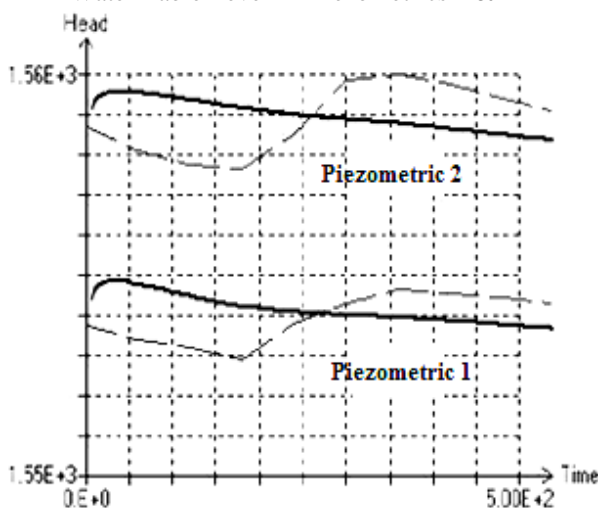


10% Decrease in Recharge Rate

Figure 15. Calculating Head for 10% Increase or Decrease in Recharge Rate in the First and Second Piezometric Wells



Water Table Level in Piezometrics 1 & 2



10% Increase in Recharge Rate

To investigate the model's sensitivity to hydraulic conductivity and specific yield parameters, each parameter was manipulated separately in the area and its effects on the water table level in the middle of the plain (piezometric well #16) and on its edge (piezometric well # 20) were studied. In this study, it was observed that hydraulic conductivity parameter, after recharge rate, has the most significant effect on the piezometric water table level.

Furthermore, As displayed in Figures 16 and 17, changing the amount of hydraulic conductivity from 6 to 0.003 m/day at piezometric wells No.16 and No. 20 has brought about changes as great as 3 meters in water table level. The model sensitivity and calculated water table level in the aquifer sides (piezometric well No.20) are more of hydraulic conductivity, which seems to be because of its effect on the recharge rate of the aquifer from this area. It is worth reminding that in all the diagrams in this article, observed water table level lines as are broken lines and calculated water table level lines are shown as bold lines.

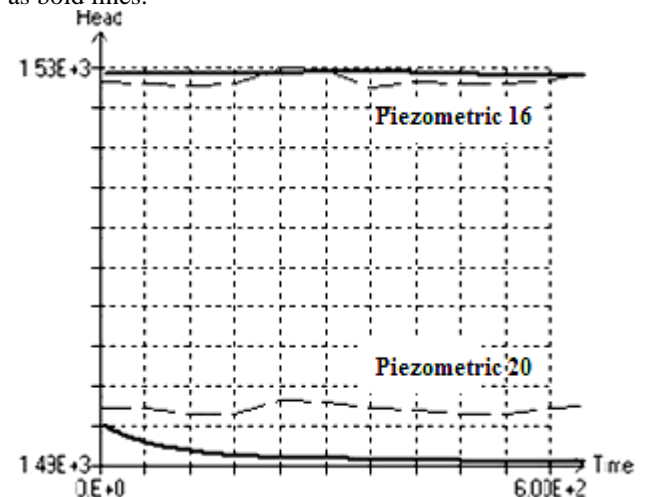


Figure 16. Piezometrics Wells 16 and 20 Water Table Level Diagram for  $k=6$  m/day

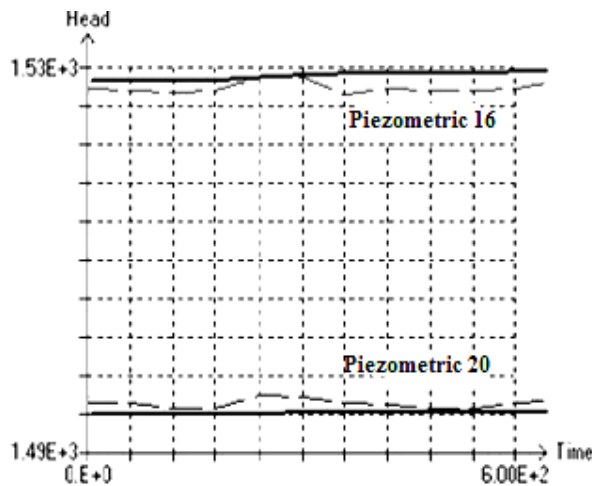


Figure 17. Piezometric Well 16 and 20 Water Table Level Diagram for  $k=0.003$  m/day

Examination of the effect of specific yield coefficient in the model showed that this coefficient exerts more effects on the ground water seasonal fluctuations in the whole plain and as is observed in Figures 18 and 19, by changing the specific yield coefficient from 0.03 to 0.15, the amount of the estimated water table fluctuations fell drastically in the whole time period. This effect is almost equal in the middle area (piezometric well No.6) and in the edges (piezometric wells No.18 and No.9).

Therefore, it can generally be concluded that the fluctuations amplitude of ground water depended on specific yield coefficient such that on the local areas where specific yield coefficient is less, fluctuation amplitude of water level will be higher.

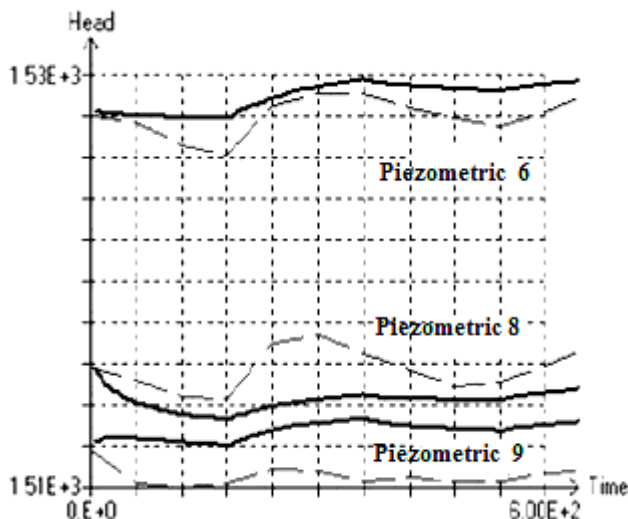


Figure 18. Estimated and Observed Water Level Diagram for  $k=6$  m/d and  $Sy=.03$

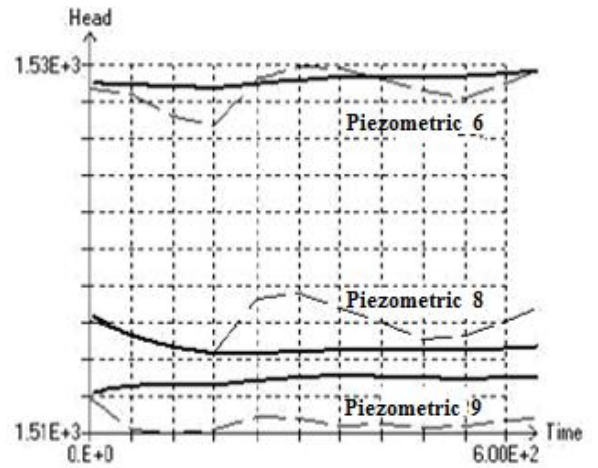


Figure 19. Estimated and Observed Water Level Diagram for  $k=6$  m/d and  $Sy=0.15$

Drain hydraulic conductance was the last parameter to which the model sensitivity was investigated. The effect of this factor on estimated water table level is shown in Figures 20 and 21. As it can be observed, increasing this coefficient by 60 times (i.e. changing drain hydraulic conductance from 0.05 to 3) brings about an insignificant change only at piezometric well No. 31 that is very close to the drain, not in two other piezometric wells.

Therefore, it could be concluded that the effect of drain hydraulic conductance is only limited to drain influence radius and it does not affect the whole model.

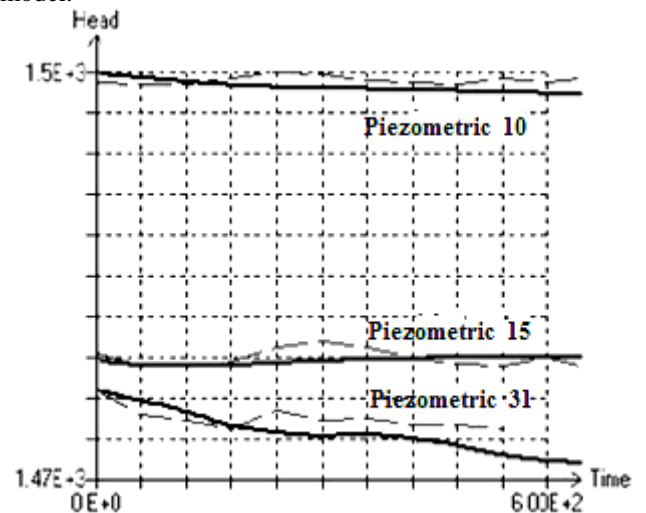


Figure 20. Estimated and Observed Water Level Diagram for  $k=4$  m/d  $Sy=0.03$ ,  $cD=0.05$  m<sup>2</sup>/d

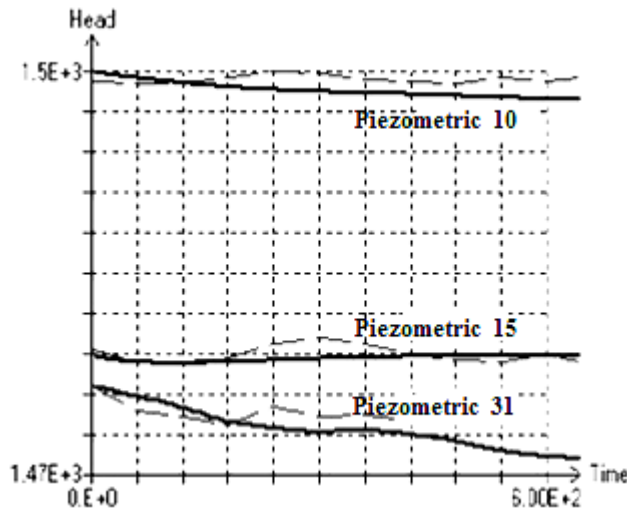


Figure 21. Estimated and Observed Water Level Diagram for  $k = 4 \text{ m/d}$ ,  $S_y = 0.03$ ,  $cD = 3 \text{ m}^2/\text{d}$

**5. Validation**

The information obtained from September 2006 to September 2007 were used for validation. The stresses placed on the aquifer were regarded as they were for the previous procedures. The results of validation have been presented in Figure 22, which shows a good fit between estimated and observed values.

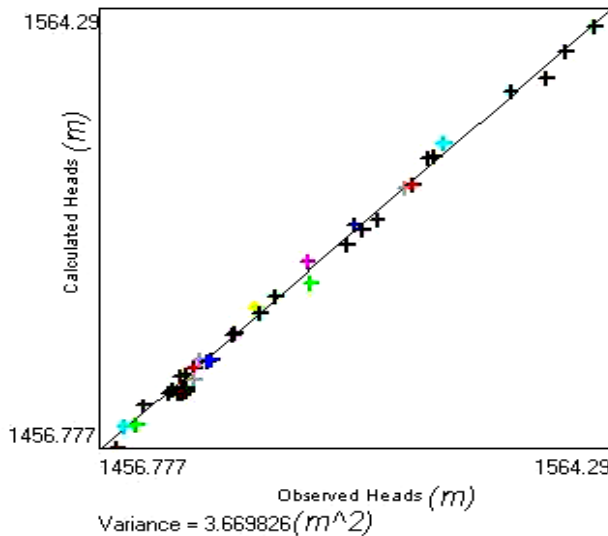


Figure 22. The Scatter Diagram and Comparison of Estimated and Observed Heads

**6. The Results of Drainage System Performance**

After model calibration, validation and specification of all the required parameters, the status of ground water level of the Shiraz plain was simulated at the end of performance of drainage in different scenarios. Scenarios considered in this case are as follows:  
 The first case: Regarding the development of the city towards Kaftarak, and the conversion of agricultural lands to residential areas, in this case agricultural pumping wells in the range of Kaftarak were off and

the ground water status of the Shiraz plain were studied with and without drainage system.

The second case: In this case, the model results were also considered with and without drainage system.

The third case: The performance of drainages and status of ground water level in drought conditions were studied.

The fourth case: The performance of drainages and status of ground water level at the end of the implementation of drainage in wet conditions were studied.

**6.1- The Results of the First Case**

Figure 23 depicts the comparison of the water table in the aquifer of the Shiraz plain with and without drainage construction. After the implementation of drainage, water table has remarkably decreased in all the plain with only a slight rise in a corner of the plain where the dormant wells of Kaftarak are located. To demonstrate the water table drawdown level, the lines in Figure 24 have been presented. As the diagram shows, the most serious reduction occurs in the northern area (the beginning of the drainage area) that is about 10 m, whereas a very low drawdown (about 0.5m) is observed at the end of the draining area near Maharloo Lake. The cause of this seems to be the change of texture of soil and its becoming fine-grained eastward, which give rise to the decrease of the radius influence of the drain as a result of the reduction of the hydraulic conductivity. Moreover, the proximity of the level drainage crossing route to the surface of the Earth has been influential in reducing the amount of drawdown in the Eastern plain. So in general, with the implementation of drainage in the case that Kaftarak pumping wells are dormant, leads to the prognosis of about 10 m drawdown at the first drainage route, and a roughly 0.5 m drawdown at the end of drainage route

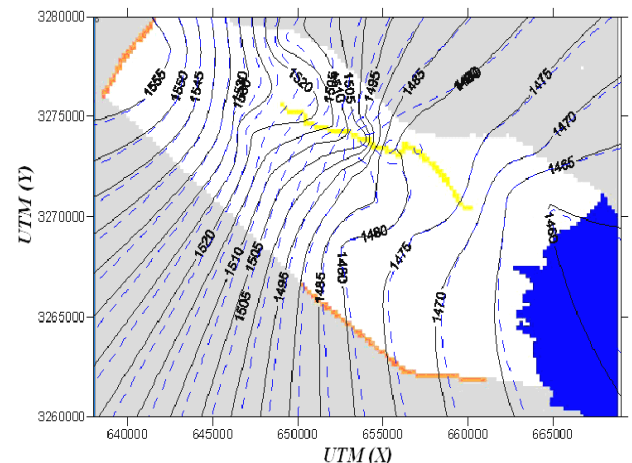


Figure 23. Comparison of Water Table in the Aquifer of the Shiraz Plain with (Bold Line) and without Drain (Broken Line)

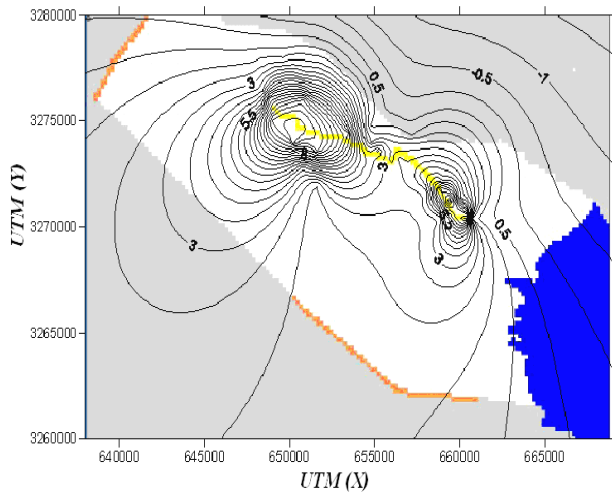


Figure 24. The Reduce Lines of Ground Water Level after the Implementation of Drainage in the First Case.

**6-2- The Results of the Model in the Second Case**

Figure 25 shows the reduction level lines of ground water after the implementation of the drainage and Figure 26 displays water table level in the aquifer of the Shiraz plain with and without drainage. As is shown, the ground water level in most of the plain zones drops and reduction of water table in more noticeable around the drainage (at about 6m). All in all, drawdown in this case is less than drawdown in the first case. And this seem logical due to the decreasing returned sewage entrance to the aquifer in the second case. Therefore, we can conclude that not only the simultaneous performance of drainage and sewage networks of Shiraz are not contradictory operations, but these plans are in fact complementary. It seems that the little increase (about 0.5m) in the ground water level in southern zones plain is due to water entrance from the southern boundary to the plain, which follows from the water level decrease in the plain.

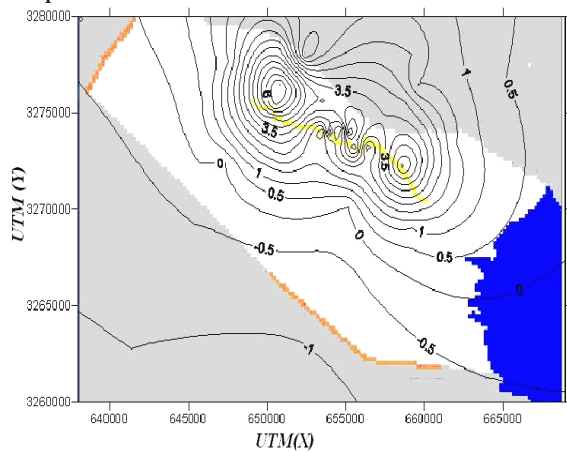


Figure 25. The Reduced depths of Ground Water Level after the Implementation of Khatoon Drainage in the Second Case.

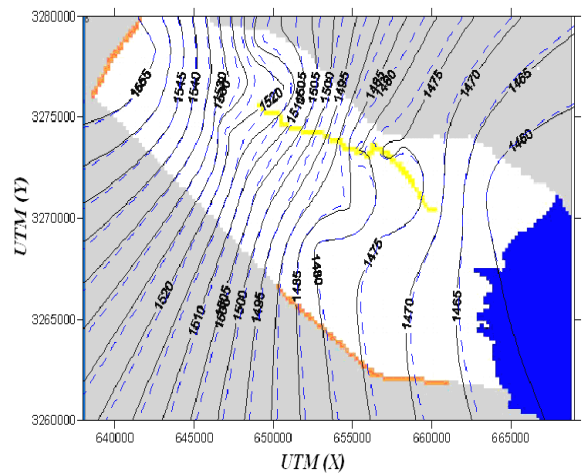


Figure 26. The Comparison of Water Table with (Bold Line) and without Drain (Broken Line.) in the Second Case

**6-3- The Results of the Model in the Third Case (Drought Conditions)**

Figure 27 shows the Reduced depths ground water level after the implementation of drainage in the drought case. In this case, we can see the reduced water level (at about 4m) and the reduction of the discharge passing through the drainage. This head loss in the plain is less than that in the second case, which seems to be logical due to the precipitation decrease. In this case, the decrease of the water level in the Maharloo Lake shore is observed. .

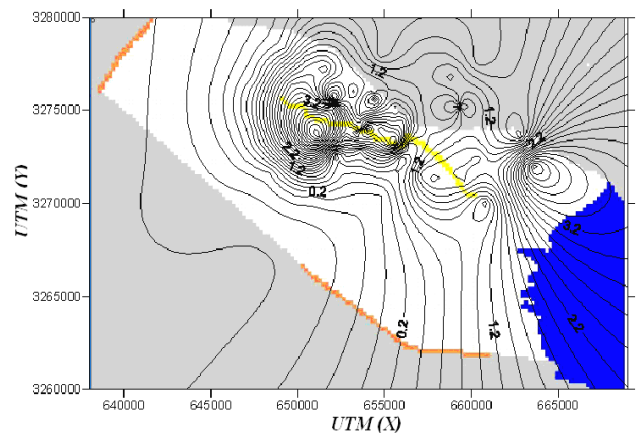


Figure 27 . The Reduced depths of Ground Water Level after the Implementation of Drainage in Third Case (drought)

**6-4- The Results of the Model in the Fourth Case (Wet Conditions)**

Figure 28 shows the reduced lines ground water level after the implementation of the drainage. As is observed in Figure 28, the water table drops down to 10 m. but in southern areas and near Maharloo Lake and northwest areas, the water table increases at about 2 m. This increase in water table can be justified owing to the increase of inflow during wet conditions, and it indicates the necessity of the implementation of the city drainage network.

Water balance in the different scenarios and the outflow rate of the drainages can help further evaluation of ground water hydraulic behavior in the different scenarios.

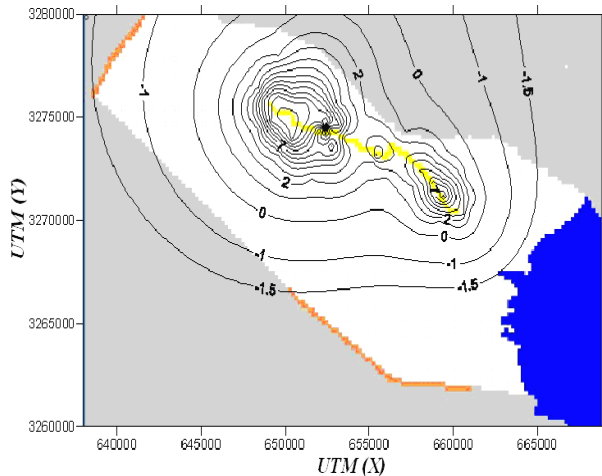


Figure 28. The Reduced depths Ground Water Level after the Implementation of the Drainage in the Fourth Case (Wet Conditions)

### 7. Water Balance in the Study Area

Using the water balance in the area, the rate of inflow from and outflow to the neighboring plains in the different scenarios were examined and compared. This comparison for the different scenarios is depicted in the Table 5.

Table 5. Inflow and Outflow Rate of Water in the Neighboring Plains for the Different Scenarios ( $m^3/d$ )

Scenario	Inflow from Western boundary	Outflow to Gharebagh plain	Outflow to Maharloo Lake	Outflow from Drainage
1-inactive Kaftarak pumping wells	1357	-2532	-2358	33117
2-implementation sewerage network	1356	-1800	-3858	31800
3-drought	406	-1000	-1360	29748
4-wet	-80	-5761	-6297	33510

The examination of the results indicates a satisfactory performance of the drainages in all the scenarios. As expected in the state of drought (scenario 3), we observe the minimum inflow and outflow and drains of the plain; and in the state of wet (scenario 4) we observe the maximum inflow and outflow and drains of the plain.

In the wet case, the flow direction reversal occurs in the plain's western boundary and a decrease in outflow from its boundary follows (due to the increase in the plain ground water). This incident is well in harmony with the rise of water table in this scenario (Figure 28). It is worth noting that the amount of flow passing through the Khatoon

drainage which was predicted by the planning consulting engineers was  $86400 m^3/day$ . Of course, in this prediction, the main drainage of Khatoon with all the branches connected to it have been investigated, and therefore according to the aforementioned figures which are calculated by the model, we can conclude that without the implementation of sub-networks, the amount of discharge passing through the drainage would be less than half of what the designers estimated.

### 8. Conclusion

Ground water hydraulic flow model of the Shiraz plain with its drainages was implemented in PMWIN software. After model calibration, validation, and specification of all the required parameters, the status of ground water level was simulated at the end of the performance of drainage and in the different scenarios.

The examination of the results implies a good performance of the drainages. This was evident in all the scenarios, where the flow passing through the drainage was more than inflow and outflow of the plain. As expected in the state of drought (scenario 3), we observed the minimum inflow and outflow and drains of the plain. However, in the state of wet (scenario 4), we observe the maximum inflow and outflow and drains of the plain.

For water level to drop down to the desired depth, minor drainage lines had to be utilized; otherwise, the amount of discharge of drainage would be less than half of what the designer estimated. In this study, hydraulic behavior of the Shiraz plain aquifer was simulated using PMWIN.

The performance of recently constructed drainage system in the plain was modeled and plain hydrodynamic coefficients were estimated via calibration, and sensitivity analysis of the model was performed for four important parameters. The results indicate that the model is sensitive to recharge rate and hydraulic conductivity, respectively. This being so, a small variation in these two parameters causes a dramatic change in hydraulic head distribution in the plain. Furthermore, specific yield coefficient influences the seasonal water level fluctuations, but the aqueducts conductance coefficient only affects the aqueduct radius of influence with little effect on the overall hydraulic behavior of the plain.

### 9. References

- [1] Bakker, M. (1999). "Simulating groundwater flow in multi-aquifer systems with analytical and numerical Dupuit models", *Journal of Hydrology*, 222, 55-64.
- [2] Anderson, E.I. (2005). "Modeling ground water-surface water interactions using Dupuit approximation", *Journal of Advances in Water Resources*, 28, 315-327.
- [3] Knowles, I. and Yan, A. (2007). "The reconstruction of groundwater parameters from head



in an unconfined aquifer", *Journal of Computational and Applied Mathematics*, 208, 72-81.

5/16/2012

[4] Mukhopadhyay, A., Al-Sulaimi, J., and Barrat, M. J. (1994). "Numerical modeling of groundwater resource management options in Kuwait." *Groundwater*, 32 (6), 917-928.

[5] Ramireddygar, S. R., Sophocleous, M. A., Koelliker, J. K., Perkins, S. P., and Govindaraju R. S. (2000). "Development and application of a comprehensive simulation model to evaluate impacts of watershed structures and irrigation water use on stream flow and groundwater: The case of wet walnut creek watershed, Kansas, USA." *J. of Hydrology*, 236 (3), 223-246.

[6] Rezaei, H., and Mousavi, S.A. (2003). "Study of Bajgah plain groundwater situation using the finite difference three dimensional modular Modflow model." *Proc. of the Second National Student Conf. on Water and Soil Resources*, Shiraz University, 907-916.

[7] Jiao, J. J., and Leaner, D. N. (1996). "Using sensitivity analysis to assist parameter zonation in groundwater flow model." *Water Resources Bulletin*, 32 (1), 75-78.

[8] Osman, Y. Z., and Bruen, M. P. (2002). "Modeling stream-aquifer seepage in an alluvial aquifer: An improved looping- stream package for MODFLOW." *J. of Hydrology*, 264, 69-86.

[9] Johannes, H. A. (2004). "Modeling water balance in Rio Turbio aquifer, Mexico." M.Sc. Thesis, Wagenin Gen University, Mexico.

[10] Samani, N., Kompani-Zare, M., and Barry, D. A. (2004). "MODFLOW equipped with a new method for the accurate simulation of axisymmetric flow." *J. of Advances in Water Resources*, 27, 31- 45.

[11] Rodriguez, L. B., Cello, P. A., Vionnet, C. A., and Goodrich, D. (2008). "Fully conservative coupling of HEC-RAS with MODFLOW to simulate stream-aquifer interactions in a drainage basin." *J. of Hydrology*, 353, 129-142.

[12] Rosemary, W. H. C., Greg, M. P., Earman, S., and Ronald, L. H. (2008). "A comparison of groundwater fluxes computed with MODFLOW and a Mixing model using deuterium: Application to the eastern Nevada test site and vicinity." *J. of Hydrology*, 361, 371- 385.

[13] Fars Regional Water Organization. (1996). Reports on Shiraz plain studies, Shiraz.

[14] Parab consulting Eng. Co. (1993). Feasibility studies on water table drawdown in southeastern Shiraz and its transfer to Sarvestan plain, Vol. 3, Shiraz.

[15] Parab consulting Eng. Co. (1993). Feasibility studies on water table drawdown in southeastern Shiraz and its transfer to Sarvestan plain, Vol. 3, Shiraz.

[16] Kresic, N. (1997). Quantitative solution in hydrology and groundwater modeling, Lewis Pub., CRC Press, Boca Raton Inc., USA.

## Current Nursing Practice for Prevention of Ventilator Associated Pneumonia in ICUs

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**Abstract:** Ventilator-associated pneumonia (VAP) continues to be an important cause of morbidity and mortality in ventilated patient. Strategies for prevention of VAP is a significant concern for health care team in intensive care units (ICUs). Nursing practice for prevention of VAP would have a significant impact on patient outcome. The main aim of the study was to investigate the current nursing practice for prevention of VAP in ICUs. The study involved a convenient sample of 150 critical care nurses. Data were collected from six ICUs at one University Hospital in Egypt between June 2011 and September 2011. Two methods were used for data collection including nurses' self administered questionnaire about the current practices for prevention of VAP, and direct observation of nursing care of mechanically ventilated patients. The results of the study revealed that there is no available protocol for VAP prevention in the studies ICUs. This explains the variation in nursing practice among ICUs, and why all evidences are not translated into practice. The findings of the study highlighted the need for developing and implementing a protocol for VAP prevention in ICUs. There is also a need for training programs for nurses on infection control and VAP preventive measures.

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**Keywords:** Ventilator associated pneumonia, intensive care units, risk of VAP, prevention.

### 1. Introduction

Ventilator-associated pneumonia is the most common intensive care unit acquired infection among patients receiving mechanical ventilation (**Rello et al., 2002; Pieracci and Barie 2007**). VAP is defined as a form of nosocomial pneumonia that occurs in patients receiving mechanical ventilation for greater than 48 hours (**Kollef, 1999**). It affects 8% to 27% of mechanically ventilated patients (**Chastre and Fagon 2002**). The rate of VAP in developing countries is higher than the National Healthcare Safety Network benchmark rates, and is associated with a significant impact on patient outcome (**Arabi et al., 2008**). The mortality rates in a patient with VAP range from 20 to 70% (**Heyland et al., 1999; Chastre and Fagon, 2002; Tejerina et al., 2006**). The predominant organisms responsible for infection are *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and Enterobacteriaceae (**Chastre and Fagon 2002**). Numerous risk factors for the development of VAP have been identified in the literature (**Bonten et al 2004; Maselli and Restrepo, 2011**). These factors are divided into modifiable and nonmodifiable (**Kollef, 1999; Tablan et al. 2004**). Modifiable risk factors involve the supine position, gastric overdistension, contamination of ventilator circuits, frequent patient transfers and low pressure of the endotracheal tube cuff. Nonmodifiable factors include male gender age over 60 years, acute respiratory distress syndrome, multiorgan failure, coma, chronic obstructive pulmonary disease, tracheostomy, re-intubation, neurosurgery and cranial trauma (**Kollef,**

**1999; Tablan et al. 2004**). Airway intubation was identified as the most important risk factor. VAP increases the duration of mechanical ventilation, prolongs ICU length of stay, increases mortality rate and increases health care costs (**Luna et al 2003; Gillespie 2009; Bonten, 2011**). The cost of VAP is approximately five-fold higher than non-infected patients (**Erbay et al, 2004**). Therefore, prevention of VAP was on the top of research agenda in intensive care medicine in the past 25 years (**Bonten, 2011**). The prevention of VAP is also a major challenge, and a significant concern for critical care nurses who care for mechanically ventilated patients. Critical care nurses have an important role in reducing risk factors, identifying early symptoms (**Myrianthefs et al., 2004**), and implementing relevant preventive measures. There is evidence that these measures decrease the incidence of VAP and improve patient outcome (**Maselli and Restrepo, 2011**). Various strategies and guidelines for prevention of VAP have been developed and recommended. The 2003 guidelines (**Tablan et al., 2004**) from The Centers for Disease Control and Prevention (CDC) in the United States of America (USA) provide recommendations for nursing practice. The CDC guideline has been implemented in the USA (**Manangan et al., 2000**). The greatest number of reports on VAP and its preventive measures in ICUs is published from USA and other European countries, but information on this area from Egypt is scarce. Hence, the main objective of this study was to investigate the current nursing

practice for prevention of VAP in ICUs at one University Hospital in Egypt.

## 2. Methodology

**Design:** This study has a descriptive design.

**Setting:** The study was conducted in six adult ICUs including surgical, general, neurological, medical, anesthesia, chest and hepatic ICUs at one University Hospital in Egypt.

**Sample:** The study included a convenient sample of 150 nurses who work in one of the studied ICUs, and who accepted to participate in the study.

**Tools:** Two tools were used for collecting data about the current practices for prevention of VAP in ICUs .

Tool 1: "**Nursing Practice for Prevention of VAP Questionnaire**"

The questionnaire was designed to investigate the measures critical care nurses use to reduce the rate of VAP. The questionnaire consisted of four sections. The first three sections were adopted from **Cason et al., (2007)** tool "The Oral Care Ventilator Patients Questionnaire" after making minor modifications. **Cason et al., (2007)** adapted this tool from **Sole et al (2001)** instrument "Techniques and Airway management Practices". The first section of the tool included questions on general characteristics of the participants: years of ICU experience, level of education, job title and the type of ICU where the participants worked. The second section involved questions about the CDC guidelines, such as the frequency of hand washing, use of gloves for oral care, subglottic suctioning, the degree of the head of the bed, and nurses' education about VAP. The third section involved questions about oral care practice (**Cason et al., 2007**). The fourth section of the questionnaire asked about the current practice for endotracheal tube route, suctioning and ventilator care. This section was developed by the investigators based upon relevant guidelines and literature (**Sole et al., 2002 1; Sole et al., 2003; Labeau et al., 2007**).

The questionnaire was translated into Arabic version by the authors. To ensure the validity of translation, back translation technique was used (**Birbili, 2000**) by a translator from the Faculty of Education, the English Department. The two versions were reviewed, and modifications were made accordingly. We pilot-tested the questionnaire between May and June 2011 in Surgical ICU and Chest ICU at one University Hospital, involving ten critical care nurses. The aim of the pilot study was to assess the clarity of the statements, and make necessary amendments prior to the main study.

Tool 2: "**Nursing Practice for Prevention of VAP Observation Form**"

This tool was developed based upon relevant guidelines and literature (**Dodek et al., 2004; Tablan**

**et al., 2004; Branson 2005; Lorente, et al.,2007; Coffin et al., 2008; Muscedere et al., 2008**). It is a checklist involved 15 items related to measures for prevention of VAP, such as decontamination of hands, patient's position, regular oral care, and sterilization of suction equipment. Investigators responded to items by checking one of two choices: "done" or "not done".

### Data collection

Data were collected between June 2011 and September 2011. Before starting the data collection process, baseline information about the studied ICUs were collected involving the number of beds in each unit, the existence of guideline for prevention of VAP, and the number of ventilated patients in each ICU. Two methods were used for data collection including nurses' self administered questionnaire about the current practices for prevention of VAP and direct observation of nursing care of mechanically ventilated patients.

Questionnaires were distributed to all critical care nurses working in the studied ICUs after explaining the aim of the study. Of the 160 distributed questionnaires, we collected 150, reflecting an overall response rate of 93.75% .

Observation of nursing care of mechanically ventilated patients were carried out between 8 am and 12 am during morning shifts. Thirty observations were collected from each ICU by the investigators. The total number of observations was 180.

### Ethical considerations

The study was approved by the ethical review committee of the Faculty of Nursing affiliated to the University from which data were collected. Permission to conduct the study was obtained from the study site administrative authorities. It was emphasized to all nurses working in the studied ICUs that participation in the study was voluntary. Verbal consent was obtained from nurses who accepted to take part in the study. In order to maintain the confidentiality of the participants, the responses were collected anonymously, data were coded, and the name of the hospital from which data were collected was not be referred to in any published work.

### Data analysis

Descriptive statistics were computed for all data. The Statistical Package for Social Science (SPSS version 15.0) was used to analyze the collected data. To explore differences in care practice among ICUs, Chi-Square test was used.

## 3. Results

### Demographic characteristics of participant nurses

Table 1 presents the demographic data of participants. The largest percentage (40.7%) had between 6 and 10 years of ICU experience, and 34% had between 1and 5 years of ICU experience. The largest percentage (40.7%) held high school level

nursing education certificate, 24.7% held a bachelor degree in nursing, and 34.7% graduates of Technical Nursing Institute. The largest percentage (39.3%) were staff nurses, 36% were Technical Institute nurses and 24.7% were head nurses. The largest percentage (33.3%) work in surgical ICU.

#### **Nurses' self report of the current general practice for VAP prevention**

Table 2 illustrates nurses' self report of general nursing practice for prevention of VAP in the studied ICUs. Most participants (53.3%) reported always washing their hands between patients and 43.3% frequently wash their hand between patients. Similarly, most participants (52%) reported always using gloves for oral care, and 46.7% reported doing it frequently. A total of 46.7% reported performing subglotting suctioning frequently, while 22.7% said that they always carry out this procedure. A total of 39.3% maintain the head of the bed elevated at 30-45 degree for a mechanically ventilated patient, 35.3% perform this procedure three times per day, 22.7% do it twice per day, and 2.7% carry out this procedure only once per day. The vast majority of participants (91.7%, 98.7% respectively) did not attend infection control workshops or conferences, or any training program on VAP prevention.

#### **Oral care practice**

Table 3 shows nurses' self report of oral care practice. According to the vast majority of participants (98.7%), no oral care protocol is available in the ICU. A total of 30% participants reported carrying out oral suctioning every 8 to 12 hours, 28% perform it every 4 hours, 26% carry out this procedure only as needed, and 15.3% did oral suctioning every 2 hours. With regard to tooth brushing, a total of 43.3% indicated carrying out tooth brushing only as needed, 33.3% provide tooth brushing every 8 to 12 hours and only 22% perform this procedure every 4 hours. Concerning oral swabbing technique, 81.3% reported carrying out oral swabbing only as needed. Most participants (72.6%) use normal saline as a mouthwash for patient's oral care, 6.7% use Hydrogen Peroxide, and 20.7% do not use any solution for oral care.

#### **Current practice for endotracheal tube route, suctioning and ventilator care**

Table 4 presents nurses' self report of the current practice of endotracheal tube route, suctioning, and ventilator care. With regard to endotracheal tube and suctioning, most participants (98.0%, 81.3%, and 98.7% respectively) reported using oral intubation route, daily changing of suction system, and using open suction system. A total of 62.0% reported changing the ventilator circuits every new patient, 26% change it only when clinically indicated. A variation in the frequency of airway humidifier use

was evident. A total of 32.7% reported sometimes using airway humidifier, 32% rarely use it, and 26% do not use it at all. The majority of participants (84.7%, 86% respectively) stated using heated humidifier, and sterile water as a solution for the airway humidifier. According to the largest percentage of participants (61.3%), airway humidifier is changed when clinically indicated.

**Table 1: Demographic characteristics of participant nurses**

Characteristics	N 150	%
<b>Years of experience</b>		
< 1 year	10	6.7
1 – 5 years	51	34
6 – 10 years	61	40.7
11 – 20 years	26	17.3
> 20 years	2	1.3
<b>Level of education</b>		
Bachelor degree	37	24.7
Technical Nursing Institute	52	34.7
High school nursing education	61	40.7
<b>Job</b>		
Head nurse	37	24.7
Technical Institute nurse	54	36
Staff nurse	59	39.3
<b>Type of Intensive Care Unit</b>		
Surgical	50	33.3
General	9	6
Neurological	12	8
Medical	12	8
Anesthesia	19	12.7
Chest	23	15.3
Hepatic	25	16.7

#### **Observations of the current practice for prevention of VAP in the studied ICUs**

Table 5 illustrates the observed current practices for prevention of VAP in the studied ICUs. Observations showed that there is no significant differences among ICUs in the use of gloves when handling body fluids ( $P=0.544$ ). However, a significant difference was noted among the studied ICUs ( $P=0.012^*$ ) concerning the decontamination of hands before caring for the patient, as most nursing staff of Chest ICU were more committed to decontamination of hands before caring for the patient than the staff in other ICUs.

**Table 2: Nurses' self report of general preventive measures for VAP**

Nursing Practice	Total	
	No.	%
<b>1- Hand washing between patients</b>		
Always	80	53.3%
Frequently	65	43.3%
Sometimes	3	3.3%
<b>2-Use of gloves for oral care</b>		
Always	78	52.0%
Frequently	70	46.7%
Sometimes	2	1.3%
<b>3- Perform subglottic suctioning</b>		
Always	34	22.7%
Frequently	70	46.7%
Sometimes	44	29.3%
Rarely	2	1.3%
<b>4- Maintains head of bed elevated at 30 - 45 degree</b>		
Once per day	4	2.7%
Twice per day	34	22.7%
Three times per day	53	35.3%
All the day	59	39.3%
<b>5- Patient's position most frequently used</b>		
Supine positioning	40	26.7%
Semi recumbent position	110	73.3%
Prone position	0	0%
<b>6- Education</b>		
<b>Attending infection control workshops and conferences</b>		
Yes	13	8.7%
No	137	91.3%
<b>Attending training programs on prevention of VAP</b>		
Yes	2	1.3%
No	148	98.7%

There was a significant difference ( $P=0.009^*$ ) concerning maintaining the patient in a semi recumbent position ( $30^\circ$  to  $45^\circ$ ) among the studied ICUs. It worth mentioning that all the staff (100%) of Hepatic and Neuro ICUs maintain patient in a semi recumbent position. Observations showed that all nurses in the studied ICUs do not use any antiseptic solution for oral care, do not maintain adequate pressure in endotracheal tube cuff, do not use closed endotracheal suctioning system, or sterilize or disinfect suction equipment. A significant difference ( $P=0.001^*$ ) was noted among ICUs concerning checking the nasogastric tube for residual volume. A statistical differences were found regarding the use of a sterile technique when applying tracheal suctioning ( $P=0.050^*$ ) and rinsing reusable respiratory equipment with sterile water ( $P=0.004^*$ ). In all the studied ICUs, a new ventilator circuit is used for each patient, and the circuits are changed when become solid or malfunctioned. Statistical significant differences were found regarding certain aspects of ventilator care, such as removing condensate from ventilatory circuit ( $P=0.014^*$ ), using sterile water for bubbling humidifier ( $P=0.000^*$ ), and changing a heat-moisture exchanger when becomes malfunction or solid ( $P=0.033^*$ ).

**Table 3: Nurses' self report of oral care practice**

Nursing Practice	Total	
	N= 150	%
<b>1- Availability of a written oral hygiene protocol in ICU</b>		
Yes	2	1.3%
No	148	98.7%
<b>2-Frequency of oral suctioning</b>		
Every 2 hours	23	15.3%
Every 4 hours	42	28.0%
Every 8-12 hours	45	30.0%
Only as needed	40	26.7%
<b>3-Frequency of tooth brushing</b>		
Every 4 hours	33	22.0%
Every 8-12 hours	50	33.3%
Only as needed	65	43.3%
Not at all	2	1.3%
<b>5- Frequency of oral swabbing</b>		
Every 4 hours	3	2.0%
Every 8-12 hours	16	10.7%
Only as needed	122	81.3%
Not at all	9	6.0%
<b>5- Mouth wash solution</b>		
Chlorhexidine Gluconate	0	0.0%
Hydrogen peroxide	10	6.7%
Others (saline)	109	72.6%
Don't use any solutions	31	20.7%

**Table 4: Nurses' self report of the current practice of endotracheal tube route, suctioning and ventilator care**

Nursing Practice	Total	
	N= 150	%
<b>Endotracheal tube and suctioning</b>		
<b>1- Endotracheal route</b>		
Oral intubation	147	98.0%
Nasal intubation	1	.7%
Both routes of intubation	2	1.3%
<b>2- Frequency of changing suction system</b>		
Daily change	122	81.3%
Weekly change	5	3.3%
Every new patient	15	10.0%
When clinically indicated	8	5.3%
<b>3- Suction system</b>		
Open suction systems	148	98.7%
Closed suction systems	2	1.3%
Both systems	0	0.0%
<b>Care of ventilator</b>		
<b>1- Frequency of ventilator circuits changes</b>		
Every 48 hours	10	6.7%
Every week	7	4.7%
Every new patient	93	62.0%
When clinically indicated	40	26.7%
<b>2- Frequency of airway humidifier use</b>		
Always	14	9.3%
Sometimes	49	32.7%
Rarely	48	32.0%
No	39	26.0%
<b>3- Type of solution used in airway humidifier</b>		
Normal saline	129	86.0%
Sterile water	17	11.3%
Taped water	4	2.7%
<b>4- Type of airway humidifier</b>		
Heated humidifier	127	84.7%
Heat and moisture exchangers	1	.7%
Both types of humidifiers	22	14.7%

5- Frequency of humidifier changes		
Every 48 hours	22	14.7%
Every 72 hours	5	3.3%
Every week	31	20.7%
When clinically indicated	92	61.3%

**Table 5: Observations of current nursing practices for prevention of VAP in ICUs**

Practices		Surgical ICU	Neuro ICU	Medical ICU	Anesthesia ICU	Chest ICU	Hepatic ICU	Test of significance	
		%	%	%	%	%	%	X <sup>2</sup>	P value
1	Wearing gloves when handling body fluids.	46.7	43.3	46.7	50	43.3	43.3	8.875	0.544
2	Decontaminating hands before caring for the patient.	76.7	73.3	86.7	86.7	90	73.3	22.755	0.012*
3	Maintaining patients in a semi recumbent position (30 to 45) unless contraindicated.	76.7	100	86.7	86.7	76.7	100	15.328	0.009*
4	Performing regular oral care with an antiseptic solution.	0	0	0	0	0	0	7.119	0.212
5	Maintain adequate pressure in end tracheal tube cuff.	0	0	0	0	0	0	1.388	0.926
6	Checking the nasogastric tube for residual volume.	16.7	30	6.7	36.7	10	13.3	28.981	0.001*
7	Performing continuous subglottic suctioning before deflating cuff or repositioning the tube.	33.3	0	6.7	26.7	23.3	13.3	32.850	0.000*
8	Using closed endotracheal suctioning system.	0	0	0	0	0	0	-	-
9	Using sterile technique when applying tracheal suctioning.	20	10	26.7	36.7	23.3	26.7	18.284	0.050*
10	Sterilization or disinfection of suction equipment.	0	0	0	0	0	0	9.874	0.079
11	Using new ventilator circuits for each patient.	100	100	100	100	100	100	-	-
12	Changing ventilator circuits when become soiled or malfunctioned.	100	100	100	100	100	100	-	-
13	Removing condensate from ventilatory circuit.	83.3	86.7	86.7	73.3	66.7	100	14.224	0.014*
14	Using sterile water to fill bubbling humidifier.	20	56.7	13.3	0	0	0	65.177	0.000*
15	Changing a heat-moisture exchanger that is in use by a patient when it malfunctions mechanically or becomes visibly soiled.	6.7	0	0	13.3	0	0	19.584	0.033*

#### 4. Discussion

The findings of nurses' self report questionnaire indicate that a large percentage of critical care nurses implemented some preventive measures for VAP. However these measures were not uniformly implemented in the studied ICUs. More than half of nurses (53.3%) reported always washing their hands, and 43.3% reported frequently washing their hands between patients. Observation of nursing care also showed that most participants are adhered to hand hygiene practice. Similarly, **Cason et al., (2007) and Grap and Munro (1997)** studies illustrated nurses' compliance with hand washing practice. Nurses' self report of the use of gloves for oral care were between 'always' (52%) and 'frequently' (46.7%) performing this practice for mechanically ventilated patients. In **Cason et al (2007)** study, most nurses reported wearing gloves in to provide oral care. The practice of hand washing and routine gloves are the most important actions taken for reducing transmission of microorganisms in ICUs (**Tablan et al., 2004**). Hence, all ICU nurses must adhere to the recommendations of hand washing and wearing gloves (**Cason et al., 2007**).

The findings of the current study showed variations in performing subglottic suctioning with a large percentage of nurses reporting 'frequently' (46.7%) carrying out this procedure and 22% reporting always performing this procedure. In **Krein et al., (2008)** study, only 21% of the surveyed hospitals reported using subglottic secretion drainage. Muscedere et al., (**2011**) meta analysis study concluded that the use of endotracheal tubes with subglottic secretion drainage is effective for the prevention of VAP, and may be associated with reduced duration of mechanical ventilation and ICU length of stay. In the same sense, **Dezfulian et al., (2005)** meta analysis study illustrated that continuous aspiration of subglottic secretions reduced the incidence of VAP by half, shortened ICU stay by 3 days, and delayed the onset of VAP by 6 days.

Semi recumbent position was the most frequently position used in the studied ICU. There is a strong evidence that placing the patient in a semi recumbent position prevents aspiration, thereby reducing the risk for VAP (**Drakulovic et al., 1999; Tablan et al., 2004**). However, the findings of a recent multicenter, observational study suggest that backrest elevation was less than recommended, and was influenced by clinical practices and patient condition (**Rose et al., 2010**). A meta-analysis conducted by **Alexiou et al., (2009)** showed that patients who were placed in a semirecumbent position at 45° have significantly

lower incidence of VAP compared to those who were placed in a supine position. Despite discrepancies in the reported evidence concerning the semi recumbent position, it remains a common VAP prevention recommendation.

The findings of nurses' self report questionnaire, and observations showed that there was no written oral care protocol available in all the studied ICUs. This is similar to the findings of a study conducted in Alexandria Main University Hospital in Egypt which reported absence of oral care protocol in the ICUs (**Alhirishi, 2010**). This actually explains the discrepancies in the practice of oral care among the studies nurses. A largest percentage of nurses reported the use of tooth brushing only as needed, and the majority reported carrying out oral swabbing only as needed. This may be due to unavailability of oral care supplies in the studied ICU. **Alhirishi (2010)** also found that oral care is carried out without the use of toothbrushing or antiseptic solutions. A randomized trial of dental brushing for VAP prevention (**Pobo et al., 2009**) illustrated that the toothbrush group and standard group had similar rates of suspected VAP (20.3% vs 24.7%;  $p = 0.55$ ). In the same sense, **Lorente et al., (2012)** found no statistical significant differences regarding the incidence of VAP between patients who received toothbrushing and those who did not receive toothbrushing. The two studies also concluded that adding toothbrushing to chlorhexidine oral care does not help to prevent VAP in mechanically ventilated patients (**Pobo et al., 2009; Lorente et al., 2012**). A randomized, controlled clinical trial was conducted by **Munro et al (2009)** to test the effects of toothbrushing and chlorhexidine in decreasing the risk for VAP in adult mechanically ventilated patients. They found that Chlorhexidine, reduced early VAP in patients without pneumonia, but the toothbrushing protocol did not have any significant effect on VAP. Despite the strong evidence which supports the effectiveness of Chlorhexidine in reducing the incidence of VAP (**Koeman et al., 2006; Munro et al., 2009; Snyders et al., 2011**), it is not used in the studied ICUs. In the current study, most nurses use saline as a mouth wash solution and 6.7% use Hydrogen Peroxide. Although normal saline is cost effective, but such use has not been thoroughly tested. Normal saline has limited use as a mouth rinse due to its tendency to cause dryness (**Bowsher et al., 1999**). Hydrogen Peroxide mouth rinse has been used untested for long time for ICU patients, and its use for oral care is still unresolved issue (**Berry et al., 2007**).

Oral intubation route is most common in the studied ICU. Evidence suggests that oral intubation is preferable as it prevents aspiration of contaminated secretions, hence reduces the incidence of VAP (**Kollef, 2004**). Almost, in all the studied ICU, open suction system is used as it is less expensive than closed suction system. Studies have found no differences in the incidence of VAP with open versus closed suction systems (**Zeitoun et al., 2003; Lorente et al., 2005; Siempos et al., 2008**). However, one experimental study conducted in causality ICU and general ICU in Alexandria University Hospital in Egypt illustrated that using closed suction system reduces the incidence of VAP by 20% in comparing with open suction system, decreases the mortality rate and the duration of mechanical ventilation (**Ba-Alwy, 2008**). However, the findings of this study can not be generalized to other ICUs in Egypt due to the small sample size.

Most nurses reported changing suction system daily. **Sole et al., (2002)** 2 found that suction devices becomes colonized with potential pathogens within 24 hours of use. Most nurses reported changing ventilator circuit every new patients and about a quarter carry out this procedure when clinically indicated. **Kollef et al., (1995)** randomized controlled trial of once-a-week circuit changes versus no ventilator circuit changes in adult ICU, illustrated that the incidence of VAP was 28.8% in patients receiving weekly circuit change and 24.5% in patient receiving no circuit change. Other studies have indicated that the frequency of ventilator circuit changes does not reduce the risk of VAP, hence this action is not recommend (**Cook et al., 1998; AARC Evidence Clinical Based Guidelines 2003**). Based on the current evidence, **Tablan et al., (2004) and Dodek et al., (2004)** recommended new circuits for each patient, and changing the ventilator circuits when visually become solid.

Most nurses reported using heated humidifier and sterile water as a solution for the humidifier. Two randomized clinical trials found no significant difference in the incidence of VAP when using heat and moisture exchanger filters compared with heated humidifiers (**Memish et al., 2001; Lacherade et al., 2005**). However, heat and moisture exchanger is preferable for use in adult ICUs as it reduces nurses workload, decreases financial cost, and provides better safety (**Memish et al., 2001**). Similar results were found by another meta analysis study (**Siempos et al., 2007**). Use of sterile water to fill the humidifier of ventilator is recommended by the Center for Disease Control's 1982 "Guideline for the Prevention of Nosocomial

Pneumonia". The findings showed that most nurses change humidifier when clinically indicated and 20.7% change it every week. Guidelines recommended changing a heat-moisture exchanger that is in use by a patient when it malfunctions mechanically or becomes visibly soiled (**Coffin et al., 2008**).

Observations illustrated that most nurses did not implemented infection control measures when handling patient's body fluids (except for Anesthesia ICU) and when applying tracheal suctioning or when dealing with suction equipment. This could be due to lack of nurses' education and training on infection control measures. **Alp and Voss (2006)** emphasized that hand washing before and after patient care, using of gloves when dealing with body fluids, and sterilizing equipment are basic elements in prevention of VAP. This indicates the need for infection control training programs for all critical care nurses working the studied ICU. It is noted that in all the studied ICUs, nurses performed oral care without any antiseptic solution, and did not use closed tracheal suctioning. As mentioned early, this is due to unavailability of supplies and inadequate resources required for carrying out these procedures. In all the studied ICUs, nurses did not maintain adequate pressure in endotracheal tube (ETT) cuff which reflects their inadequate knowledge about the importance of this action in prevention of VAP. Maintaining the ETT cuff pressure between 20 and 30 cm H<sub>2</sub>O prevents the movement of secretions from the mouth into the lungs, hence reduces the incidence of VAP (**Grap et al, 2012**). Significant differences were found among the studied ICUs concerning some aspects of care, such as checking nasogastric tube for residual volume, performing subglottic suctioning before deflating cuff, using sterile technique when applying tracheal suctioning and using sterile water to fill bubbling humidifier. Variations in nursing practice among ICUs, and not implementing all evidences into practice could be due to the absence of a uniform protocol for VAP prevention in the studied ICU, and lack of nurses' training in this area. Hence, in order to decrease the incidence of VAP, protocols for VAP prevention and monitoring tools must be developed (**Augustyn, 2007**). Additionally, critical care nurses need to receive training on VAP preventive measures and to be orientated about the current evidence based guidelines. **Babcock et al (2004)** found that an educational program for ICU nurses and respiratory therapy staff on correct practices for VAP prevention, risk factors and preventive strategies was associated with reduced rate of VAP in ICU setting. In the same sense, **Gallagher's (2012)**



study findings suggest that education of nurses can improve mechanically ventilated patient outcome, and improve the quality of care.

On the other hand, it is very interesting that in the absence of VAP prevention protocol, and infection control training programs at the hospital, participant nurses implemented some of the preventive measures, and followed some of the recommendations for VAP prevention. May be nurses leaned what they know from their clinical experience, or may be from doctors' instructions. This issue worth to be investigated.

### Conclusion and recommendations

The current study provided a rounded picture of the current nursing practice for prevention of VAP in ICUs. The findings revealed variations in nursing practice for VAP prevention across the studied ICUs. The study illustrated an absence of a uniform protocol for prevention of VAP in the studied ICU. This indicates the need for developing a protocol for VAP prevention based upon current evidence based guidelines. There is also a need for establishing a system to ensure that VAP prevention protocol will be implemented consistently in all ICUs. We recommend involving all ICUs nurses in infection control training programs and VAP prevention program to update their knowledge and enhance their skills in this area. For newly employed staff, we recommend integrating infection control and VAP prevention programs as a part of critical care orientation program. Hospital administrative authorities should provide the supplies and resources required for prevention of VAP. Further qualitative studies are required to explore the basis of nursing practice for prevention of VAP in ICUs.

### Limitations

Our findings represent nursing practices for prevention of VAP in six ICUs at one University Hospital in Egypt. Hence we can not make generalization from the current study.

### References

1. AARC Evidence-Based Clinical Practice Guidelines: Care of the ventilator circuit and its relation to ventilator-associated pneumonia (2003). *Respir Care*, 48:869-879. <http://www.ann-clinmicrob.com/content/5/1/7>
2. Alexiou V, Ierodiakonou V, Dimopoulos G, Matthew F (2009). Impact of patient position on the incidence of ventilator-associated pneumonia: A meta-analysis of randomized controlled trials. *Journal of Critical Care*, 24(4): 515-522.
3. Alhirishi M (2010). Critical care nurse application of evidence based guidelines for preventing ventilator associated pneumonia. MSc, University of Alexandria, Egypt.
4. Alp E, Voss A (2006). Ventilator associated pneumonia and infection control. *Annals of Clinical Microbiology and Antimicrobials*, 5:7. <http://www.ann-clinmicrob.com/content/5/1/7>
5. Arabi Y, Al-Shirawi N, Memish Z, Anzueto (2008). A Ventilator-associated pneumonia in adults in developing countries: a systematic review. *International Journal of Infectious Diseases*, 12: 505—512.
6. Augustyn B (2007) Ventilator-associated pneumonia: Risk factors and prevention. *Crit Care Nurse* 2007;27:32-39.
7. Ba-Alwy A (2008). Impact of closed versus open tracheal suction on the occurrence of ventilator associated pneumonia. PhD Thesis, University of Alexandria, Egypt.
8. Babcock H, Zack J, Garrison T, Trovillion E (2004). An educational intervention to reduce ventilator associated pneumonia in an integrated health system: a comparison of effects. *Chest*, 125: 2224-2231.
9. Branson R (2005). The Ventilator Circuit and Ventilator-Associated Pneumonia. *Respiratory Care*, 50(6):774–785.
10. Berry A, Davidson P, Masters J, Rolls K (2007). Patients receiving mechanical ventilation systematic literature review of oral hygiene practices for intensive care. *Am J Crit Care*;16:552-562.
11. Birbili, M., (2000). Translating from one language to another. *Social Research Update*. Issue 31, University of Surrey.
12. Bonten M (2011). Healthcare epidemiology: Ventilator-associated pneumonia: preventing the inevitable. *Clinical Infectious Diseases*, 52(1):115-21.
13. Bonten M, Kollef M, Hall J (2004). Risk factors for ventilator-associated pneumonia: from epidemiology to patient management. *Clinical Infectious Diseases* 2004; 38:1141–9.
14. Bowsher J, Boyle S, Griffiths J (1999). A clinical effectiveness systematic review of oral care. *Nurs Stand*, 13(37):31-33.
15. Cason C, Tyner T, Saunders S, Broome L (2007). Nurses' implementation of guidelines for ventilator-associated pneumonia from the centers for disease control and prevention. *American Journal of Critical Care*, 16(1): 28-36.
16. Chastre J, Fagon J (2002). Ventilator associated pneumonia. *Am J Respir Crit Care Med*. 165:867-903.
17. Coffin S, Klompas M, Classen D, Arias K; Podgorny K, Anderson D, Burstin H, Calfee D, Dubberke E, Fraser V, Gerding D, Griffin F,

- Gross P, Kaye K, Lo E, Marschall J, Mermel L, Nicolle L, Pegues D, Perl T, Saint S, Salgado C, Weinstein R, Wise R, Yokoe D (2008).
18. Strategies to prevent ventilator associated pneumonia in acute care hospitals. *Infection Control and Hospital Epidemiology*, 29: 31-40.
  19. Cook D, De Jonghe B, Brochard L, Brun-Buisson C (1998). The influence of airway management on ventilation associated pneumonia: Evidence from randomized trials. *JAMA*, 279:781-787.
  20. Dezfulian C, Shojania K, Collard H, Kim H, Matthay M, Saint S (2005). Subglottic secretion drainage for preventing ventilator-associated pneumonia: a meta-analysis. *Am J Med*, 118:11-18
  21. Dodek P, Keenan S, Cook D, Heyland D, Jacka M., Hand L, Muscedere J, Foster D, Mehta N, Hall R, Brun-Buisson C (2004). Evidence-based clinical practice guideline for the prevention of ventilator-associated pneumonia. *Ann Intern Med*, 141(4):305-313.
  22. Drakulovic M, Torres A, Bauer T, Nicolas J (1999). Supine body position as a risk factor for nosocomial pneumonia in mechanically ventilated patients: a randomised trial. *Lancet*, 354:1851-1858.
  23. Erbay R, Yalcin A, Zencir M, Serin S, Atalay H (2004) Costs and risk factors for ventilator-associated pneumonia in a Turkish University Hospital's Intensive Care Unit: A case-control study. *BMC Pulmonary Medicine*, 4:3.
  24. This article is available from: <http://www.biomedcentral.com/1471-2466/4/3>
  25. Gallagher J (2012) Implementation of ventilator associated pneumonia clinical guideline (bundle). *The Journal for Nurse Practitioners*, 8(5): 377-382.
  26. Gillespie R (2009). Prevention and management of ventilator-associated pneumonia: the care Bundle approach. *ASJCC*, 25 (2): 44-51.
  27. Grap M, Munro C (1997). Ventilator associated pneumonia: clinical significance and implications for nursing. *Heart and Lung*, 26:419-429.
  28. Grap M, Munro C, Unoki T, Hamilton A, ward K (2012). Ventilator associated pneumonia: the potential critical role of emergency medicine in prevention. *The Journal of Emergency Medicine*, 42(3):353-362.
  29. Heyland D, Cook D, Griffith L, Keenan S, Burn Buisson C (1999). The attributable morbidity and mortality of ventilator associated pneumonia in the critically ill patient. The Canadian Critical Trials Group. *Am J Respir Crit Care Med*, 159:1249-1256.
  30. Muscedere J, Rewa O, Mckechnie K, Jiang X, Laporta D, Heyland D (2011) Subglottic secretion drainage for the prevention of ventilator-associated pneumonia: A systematic review and meta-analysis. *Critical Care Medicine*, 39(8): 1985-1991.
  31. Koeman M, Van der Ven A, Hak E, Joore H (2006) Oral decontamination with Chlorhexidine reduces the incidence of ventilator-associated pneumonia. *Am J Respir Crit Care Med*, 173: 1348-1355.
  32. Kollef M (2004). Prevention of hospital-associated pneumonia and ventilator-associated pneumonia. *Crit Care Med*, 32(6):1396-405.
  33. Kollef M, Shapiro S, Fraser V, Silver P, Murphy D, Trovillion E, Hearn M, Richards R, Cracchilo L, Hossin L (1995). Mechanical ventilation with or without 7-day circuit changes: a randomized, controlled trial. *Ann Intern Med*, 123(3):168-174.
  34. Kollef M (1999). The prevention of ventilator associated pneumonia. *N Engl J Med*, 340(8):627-634.
  35. Krein S, Kowalski C, Damschroder L, Forman J, Kaufman S, Saint S (2008). Preventing ventilator associated Pneumonia in the United States: a multicenter mixed study. *Infection Control and Hospital Epidemiology*, 29(10): 933-936.
  36. Labeau S, Vandijck D, Claes B, Van Aken P, Blot S (2007). Critical care nurses' knowledge of evidence-based guidelines for preventing ventilator-associated pneumonia: An evaluation questionnaire. *Am J Crit Care*, 16:371-377
  37. Lacherade J, Auburtin M, Cerf C, Van de Louw A, Soufir L, Rebufat Y, Rezaiguia S, Lellouche F, Brun-Buisson C, Brochard L (2005). Impact of humidification systems on ventilator-associated pneumonia: A randomized multicenter trial. *American Journal of Respiratory and Critical Care Medicine*, 172: 1276- 1282.
  38. Lorente L, Blot S, Rello J (2007). Evidence on measures for the prevention of ventilator-associated pneumonia. *Eur Respir J*, 30(6): 1193-1207.
  39. Lorente L, Lecuona M, Jiménez A, Palmero S, Pastor E, Lafuente N, Ramos M, Mora M, Sierra A (2012). Ventilator-associated pneumonia with or without toothbrushing: a randomized controlled trial. *Eur J Clin Microbiol Infect Dis*, 2012 Mar 16. [www.ncbi.nlm.nih.gov/pubmed](http://www.ncbi.nlm.nih.gov/pubmed).
  40. Lorente L, Lecuona M, Martín M, García C, Mora M, Sierra A (2005). Ventilator-associated pneumonia using a closed versus an open tracheal suction system. *Crit Care Med*, 33(1):115-9.
  41. Luna C, Blanzaco D, Niederman M, Matarucco W, Baredes N, Desmery P, Palizas F, Menga G, Rios F, Apezteguia C (2003). Resolution of ventilator associated pneumonia: prospective evaluation of the clinical Pulmonary Infection score as an early clinical predictor of outcome. *Critical Care Medicine*, 31:676-682.
  42. Manangan L, Banerjee S, Jarvis W (2000). Association between implementation of CDC recommendations and ventilator-associated

- pneumonia at selected US hospital. *American Journal of Infection Control*, 28(3):222-227.
43. Maselli D, Restrepo M (2011). Strategies in the prevention of ventilator-associated pneumonia. *Therapeutic Advances in Respiratory Disease*, 5(2): 131-141.
  44. Memish Z, Oni G, Djazmati W, Cunningham G, Mah M (2001). A randomized clinical trial to compare the effects of a heat and moisture exchanger with a heated humidifying system on the occurrence rate of ventilator-associated pneumonia. *Am J Infect Control*, 29:301-305.
  45. Munro C, Grap M, Jones D, McClish D (2009). Pneumonia in critically ill adults Chlorhexidine, toothbrushing, and preventing ventilator-associated. *American Journal of Critical Care*, 18:428-438.
  46. Muscedere J, Dodek P, Keenan S, Fowler R, Cook D, Heyland D (2008). Comprehensive evidence-based clinical practice guidelines for ventilator-associated pneumonia: prevention. *Journal of Critical Care*, 23:126-137.
  47. Myrianthefs P, Kalafati M, Samara I, Baltopoulos G (2004). Nosocomial pneumonia. *Critical Care Nursing Quarterly*, 27(3):241-57.
  48. Pieracci F and Barie P (2007). Strategies in the prevention and management of ventilator-associated pneumonia. *The American Surgeon*, 73 (5): 419-432.
  49. Pobo A, Lisboa T, Rodriguez A, Sole R, Magret M, Trefler S, Gómez F, Rello J (2009). A randomized trial of dental brushing for preventing ventilator-associated pneumonia. *Chest*, 136(2):433-9.
  50. Rello J, Ollendorf D, Oster G, Vera-Llonch M, Bellm L, Redman R, Kollef M (2002). Epidemiology and outcomes of ventilator-associated pneumonia in a large US database. *Chest*, 122:2115-2121.
  51. Rose L, Baldwin I, Crawford T, Parke R (2010). Semirecumbent positioning in ventilator-dependent patients: A multicenter, observational study. *Am J Crit Care*, 19:100-108.
  52. Siempos I, Vardakas K, Falagas M (2008). Closed tracheal suction systems for prevention of ventilator-associated pneumonia. *British Journal of Anaesthesia*. This article is available from: <http://bja.oxfordjournals.org>.
  53. Siempos I, Vardakas K, Kopterides P, Falagas M (2007). Impact of passive humidification on clinical outcomes of mechanically ventilated patients: A meta-analysis of randomized controlled trials. *Critical Care Medicine*, 35(12):2843-51.
  54. Snyders O, Khondowe O, Bell J (2011) Oral chlorhexidine in the prevention of ventilator associated pneumonia in critically ill adults in the ICU: A systematic review. *SAJCC*, 27(2): 48-56.
  55. Sole L, Byers F, Ludy E (2001) STAMP survey: Suctioning techniques and airway management practices. Orlando, Fla: University of Central Florida, School of Nursing. Cited in Cason C, Tyner T, Saunders S and Broome L. (2007) Nurses' implementation of guidelines for ventilator-associated pneumonia from the centers for disease control and prevention. *American Journal of Critical Care*, 16(1): 28-36.
  56. Sole M 1, Byers J, Ludy J, Ostrow L (2002). Suctioning techniques and airway management practices: Pilot study and instrument evaluation. *Am J Crit Care*, 11:363-368
  57. Sole M , Byers J, Ludy J, Zhang Y, Banta C, Brummel K (2003). A multisite survey of suctioning techniques and airway management practices. *Am J Crit Care*, 12:220-230.
  58. Sole M 2, Poalillo E, Byers J, Ludy J (2002). Bacterial growth in secretions and on suctioning equipment of orally intubated patients: a pilot study. *Am J Crit Care*, 11:141-149.
  59. Tablan O, Anderson L, Besser R, Bridges C, Hajjeh R (2004). Guidelines for preventing health-care-associated pneumonia, 2003: recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee. *MMWR Recomm Rep*, 53(RR-3):1-36.
  60. This article is available from: <http://www.cdc.gov/mmwr/preview/mmwrhtml>.
  61. Tejerina E, Frutos-Vivar F, Restrepo M, Anzueto A, Abroug F, Palizas F, González M, D'Empaire G, Apezteguía C, Esteban A (2006). Internacional mechanical ventilation study group incidence, risk factors, and outcome of ventilator-associated pneumonia. *J Crit Care*, 21:56-65.
  62. Zeitoun S, de Barros A, Diccini S (2003). A prospective, randomized study of ventilator-associated pneumonia in patients using a closed vs open suction system. *J Clin Nurs*, 12(4):484-489.

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**Epidemiology of mental disorders among adolescents in the city of Bandar Abbas, Iran, in 2012**Azita amirfakhræi<sup>1,3</sup>, Afsaneh alinaghizadeh<sup>2</sup><sup>1</sup> Lecturer, Department of Education, Islamic Azad University, Bandar Abbas Branch, Bandar Abbas, Iran<sup>2</sup> Lecturer, Department of Education, human science faculty, Baft Branch, Islamic Azad University, Baft, Iran<sup>3</sup> Administer student counsel center, Islamic Azad University, Bandar Abbas branch, Bandar Abbas, Iran[afakhræi2002@iauba.ac.ir](mailto:afakhræi2002@iauba.ac.ir)

**Abstract:** Mental disorders are a very common health problem among adolescents. The purpose of this study was to determine the prevalence of mental disorders in high school adolescents in the city of Bandar Abbas in the 2012 academic year. In this study, the cluster sampling method was used to select 800 subjects from the student population of the high school, and a survey of their mental health was conducted by using the SCL-90-R questionnaire. The survey results were used to identify students who were suspected of having a mental disorder, and those students were evaluated using an interview method based on DSM-IV. The results showed that 16.7% of the 800 students included in the study had some mental disorder. Anxiety and mood disorders were the most prevalent mental disorders observed, with prevalence percentages of 85% and 39%, respectively. Among the demographic variables, 22% of the girls and 11% of the boys had some disorder. There were significant relationships between the mental disorders of the adolescents and a number of variables, including a family history of mental disorders, the occurrence of divorce in their families, drug addiction, and alcohol consumption. Mental disorders in the city of Bandar Abbas were more prevalent among adolescent girls than boys, and specific anxiety disorders, such as anxiety, obsessive-compulsive disorder, and depression, occurred more frequently than other mental disorders. Mental disorders were more prevalent among students from families in which there was a family history of mental disorders, divorce, drug addiction, and alcohol consumption.

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**Keywords:** Mental disorder; Adolescents; Bandar Abbas; Iran

### 1. Introduction

The health of the population is one of the most important and fundamental issues in all countries, and three perspectives of health must be considered, i.e., physical, psychological, and mental health (Omidi et al., 2004). Mentally healthy people are those who are comfortable with themselves and their surroundings. The attributes of people who are mentally healthy include their abilities to enjoy life, control their behavior, make correct assessments of factual information, work effectively, and have a sense of well-being (Carson, 2002). However, in many countries, mental health has received very little attention because health priorities have been focused on such issues as infectious and contagious diseases (Sadeghi-Movahed et al., 2009). However, mental disorders are serious and common health problems, and, worldwide, they comprise almost 20% of total referrals to healthcare centers. The published statistics related to the prevalence of mental disorders in many countries around the world, including Iran, clearly indicate that mental illnesses should be considered as an important health priority. In 2002, the World Health Organization (WHO) announced that about 500 million people worldwide are suffering from various mental disorders, with about

half of them suffering from comparatively mild mental disorders, such as depression and anxiety (Hashemi-Nazari et al., 2008). It should be noted that many of the mental disorders in adulthood are, in fact, just continuations of disorders that originated during childhood and adolescence (Kaplan Sadock, 1998). Epidemiological studies of mental disorders that have been conducted in Iran have produced varying results, with estimates of the affected population ranging from about 12% to about 30% (Mohammadi, Davidian, and Noorbala, 2004; Noorbala, Mohammadi, and Bagheri Yazdi, 2000). According to research conducted by Ehsan Mannesh (2002), the prevalence of these disorders in Iran was in the range of 18% to 23%. Our review of the previous studies performed in Iran showed that the prevalence of mental disorders was increasing over time. For example, the prevalence of mental disorders in the rural population of Meybod, Yazd was estimated to be 16.5% (Bagheri-Yazdi, 1995), while, in a study conducted by Fayegh-Yousefi et al. (2001), the prevalence of mental disorders in male and female students in Kurdistan was reported to be 37.2%. Mousavi et al. (2000) reported that the prevalence of depression 15-17 year-old students in the high schools in Abbas was 44.5%, while Hosseini

et al. (2001) reported that the prevalence of mental disorders in students at the Mazandaran University of Medical Sciences was 51.8%. Considering that the adolescent years are when personal and social structure behaviors are formed, mental disorders that occur at this time can have serious and adverse effects on a person's ultimate capabilities, which determine her or his future and destiny. Hence, paying attention to the emotional and spiritual needs of adolescents is significantly important. In addition to our efforts to provide good healthcare and educational opportunities for our children, we also must understand the factors that can harm their mental and spiritual development and develop good solutions for preventing and compensating for these factors. To accomplish this, perhaps the first step is to study the prevalence of disorders and psychopathology so that we can make adolescents aware of these challenges and how to deal with them when they occur (Robert, 1998). Using this approach, we can assist the education system

in planning for the future of the adolescents and minimize the social and psychological damages that can occur when childhood and adolescent disorders are not treated properly, allowing them to continue into adulthood. In this way, the effects of mental health issues could be reduced throughout our society. The city of Bandar Abbas was chosen as the site for the assessment of the mental health of high school adolescents due to the special features associated with the city, such as being a coastal, border city; the influx of immigrants; and the hot and humid climate.

The purpose of this research was to study the prevalence of mental disorders in high school students of the city of Bandar Abbas, and the specific objectives of this study were:

1. To determine the prevalence of mental disorders in the adolescents of Bandar Abbas in the 2012 academic year
2. To determine the highest and lowest prevalence of mental disorders among the adolescents in the high school at Bandar Abbas during the 2012 academic year
3. To compare the prevalence of mental disorders among the adolescent girls and the adolescent boys in the high school at Bandar Abbas in the 2012 academic year.

## 2. Material and Methods

The study presented in this paper was a descriptive research study. The statistical population was all of the high school students in the city of Bandar Abbas. The subjects were selected from all of the high school students in BandaAbbas during the 2012 academic year. The multi-stage cluster random

sampling method was used to select 800 subjects, 41% of whom were males and 59% of whom were females. Initially, 814 subjects were selected, but 14 were excluded, leaving a total of 800 subjects who participated in the mental health assessment.

### 2.1. Research tool

The following tools were used in the study:

#### 2.1.1. The SCL-90-R questionnaire

The Symptom Check List-90-Revised (SCL-90-R) questionnaire contains 90 questions that were used to assess the psychological symptoms reported by the subjects. The questionnaire contains nine complaints aspects, including somatization disorder, obsessive-compulsive disorder, interpersonal sensitivity disorder, depression disorder, anxiety disorder, aggression disorder, phobic anxiety disorder, paranoid personality disorder, and psychotic disorder. Each of the test materials was answered using a five-option scale, and the time allowed to complete the test was 12-15 minutes. Scoring and interpretation of the test were performed based on the Global Severity Index (GSI), Positive Symptom Distress Index (PSDI), and Positive Symptom Total (PST). The validity of the design of this questionnaire showed that it can be used effectively as a tool to diagnose mental disorders (Hosseinfard et al., 2005).

#### 2.1.2. Clinical interview

During the screening phase performed by the SCL-90-R questionnaire, 176 subjects who were suspected of having mental disorders were identified. In the second phase, a clinical interview was conducted with the subjects using the symptoms recording form based on DSM-IV diagnostic criteria, and a psychiatrist made a diagnosis based on the symptoms recorded on the form. Of the 176 subjects who were suspected of having mental disorders, 155 were interviewed, 17 of which were diagnosed as not having any mental disorder. Twenty-one subjects were not interviewed for various reasons. The data were analyzed using descriptive statistical methods and the chi-squared test.

## 3. Results

As can be seen in Table 1, 31.2% of the subjects with mental disorders lived with family members who abused the use of drugs, and 29.9% of the subjects lived with family members who abused the use of alcohol. According to our findings, nearly half of the subjects lived with both parents, and a smaller percentage lived with other relatives, e.g., in a room in their grandfather's house. In Table 2, the standard deviation and the mean GSI score on the SCL-90-R questionnaire are shown by gender.

Collectively, 16.7% of the 800 subjects had mental disorders. The disorder with the highest prevalence was anxiety, with 85% of the subjects being so diagnosed, and this disorder was observed to a greater extent among girls than among boys. The

anxiety disorder and obsessive-compulsive disorder were diagnosed more than the others, with phobic anxiety disorder occurring less frequently than the other disorders.

**Table 1.** Demographic of students

Variables	Percent			
	Male		Female	
Sexuality	340 (42.5 %)		460(57.5 %)	
Live with parent	Death one of the parent	Live with two parent	Death parent	Divorce
	14	54	8	24
Situation house	Personal house		Rental house	
	61.9		38.1	
History of mental disorder in families	Yes		No	
	29.9		70.1	
History of drug and alcohol abuse in families	Yes		No	
	31.2		68.8	

**Table 2.** The standard deviation and the mean GSI score

Scales	Female (460)		Male (340)	
	Mean	Std.Deviation	Mean	Std.Deviation
Somatization	39%	43%	29%	33%
Obsessive-compulsive	52%	50%	38%	41%
Interpersonal sensitivity	72%	67%	42%	46%
Depression	59%	57%	31%	39%
Anxiety	50%	59%	29%	40%
Aggression	47%	55%	42%	52%
Paranoididea	73%	69%	63%	57%
Phobic anxiety	28%	40%	15%	28%
Psychoticism	42%	52%	32%	43%
GSI	51%	45%	34%	33%

#### 4. Discussions

In our study of subjects who were 15 and older, the prevalence mental disorders was higher than that reported in earlier research (Afshari et al., 1998; Fones et al., 1998; Bagheri-Yazdi et al., 1995; Hosseini et al., 1995; Kokabeh, 1994; Javidi, 1994; Weissman, 1978). However, some other studies reported the prevalence of mental disorders among such adolescents to be lower than our findings (Kessler, 1994; Stansfeld, 1991; Medianos, 1987; Hoper et al., 1979). In the researcher's opinion, these differences could be due to one or more of the following:

- Using research tools other than the SCL-90-R questionnaire for assessing mental health
- Differences in sampling methods
- Differences in the ages of the subjects studied
- Differences in cultural, social and welfare characteristics

For example, in the city of Bandar Abbas, the reasons for the difference could be due to the specific characteristics of this city, such as high temperature and high humidity throughout most of the year, limiting interactions with the natural environment; lack of proper recreational and welfare facilities; the influx of immigrants; being away from family members; its status as a coastal and border city; adverse economic conditions of most families and adolescents; small, crowded houses; and limited interactions with other people. Based on our findings, the prevalence of mental disorders in the girls was twice that among the boys. This finding was consistent with the findings of earlier research conducted in Iran (Bagheri-Yazdi, 1995; Khosravi, 1995; Afshari-Monfared, 1998; Noorbala, 2002; Mohammadi, 2002; Hosseinifard, 2005). Hollifield et al. (1990) and Bahar et al. (1992) obtained similar results in studies outside of Iran, and it seems that biological factors, hot weather, social limitations, and environmental stresses are the underlying factors that

cause the higher prevalence of mental disorders in girls. Regarding the relationship between mental illness in adolescents and various factors, such as a history of psychiatric disorders in the family, substance abuse, alcohol abuse, and divorce, other researchers also have reported similar findings (Hosseinfard et al., 2005; Mohammadi, 2002; Doherty et al., 1991). One main limitation of this study was the lack of knowledge among some subjects that made it difficult for them to read the questions, understand what was being requested, and interact productively with the research staff. Another main limitation was the lack of cooperation exhibited by some of the subjects. Also, the research was expensive and consumed a lot of time because of its magnitude, i.e., the inclusion of 800 subjects. The results of his study showed that mental disorders are more prevalent in girls than in boys. The results also showed that anxiety disorders, such as disseminated anxiety disorder and obsessive-compulsive disorder, followed closely by depression disorder, are more prevalent than other mental disorders. Based on the results obtained and our analysis of those results, it is recommended that the research be repeated in mountainous areas, regions with good climates, and among other cultures using the same research tools and sampling methods so that comparisons of the factors that may affect the prevalence of mental disorders can be made.

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#### References

1. Afshari\_Monfared, Jaleh; Bolhari, Jafar; Bagheri-Yazdi, Seyed Abbas (1998). Epidemiological study of mental disorders among patients referred to public clinics in Semnan; *Teb&Tazkieh Journal*, No. 26, pp.15-10.
2. Baha, E., Henderson, A.S., &Mackinon, A.J. (1992). An epidemiological study of mental health and socioeconomic conditions in Sumatra, Indonesia. *ActaPsychiatrica, Scandinavica*, 85,257-263.
3. Bagheri-Yazdi, Seyed Abbas; Bolhari, Jafar; Shah Mohammadi, Davood (1995). Epidemiological study of mental disorders in rural areas of Meybod, Yazd; (*Andisheh&Raftar*) *Thinking & Behavior Quarterly Journal*, Year I, No. 1, pp. 32-42.
4. Bahadorkhan, Javad. (1994). Epidemiological study of mental disorders in rural areas of Gonabad; *Clinical Psychology*, Master's thesis, Psychiatric Institute of Tehran.
5. Carson, B,V (2002). *Mental health nursing* (2 nd, ed) Philadelphia: Saunder
6. Ebrahimi, Ali (1997). Epidemiological study of harassment behavior and attention deficit disorders in high school students in the city of Esfarrayen; *Clinical Psychology*, Master's thesis, Psychiatric Institute of Tehran.
7. Ehsanmanesh, Mojtaba (2002). Epidemiology of mental disorders in Iran; *Review of some conducted studies*; (*Andisheh&Raftar*) *Thinking & Behavior Quarterly Journal*, Year VI, No. 4, pp. 54-69.
8. FaeghYousefi; Erfani; Kheirabadi; Ghanei; (2001). Review of prevalence of disobedience conduct disorder in middle school students in Kurdistan Province; (*Andisheh&Raftar*) *Thinking & Behavior Quarterly Journal*, Year II & III, Autumn and Winter, 2001, pp. 48-54.
9. Fones, C.S.,Kau, E.H., &Ko, S.M. (1998). Studying the mental health of Singapor. *Singapor Medical Journal*, 53, 251-260.
10. HashemiNazari, Saeed. et al (2008). Study of mental health of Fire agencies staff using the 28 question General Health Questionnaire; *Hakim Research Journal*, Volume X, Issue II, pp. 56-64.
11. Hoeper, E.W.,Nycz, G.R., Cleary, P.D., Regier, D.A., &Golderg, I.D.(1979). Estimated prevalence of RDE mental disorder in primary care, *International Medical Journal of Mental Health*, 1979, 8-15.
12. Hollofield, M., Laton, W., Spain, D., & Pule, L. (1990). Anxiety and depression in a village of Lesotho: A comparison with the United States. *British Journal of Psychiatry*, 156, 343 – 350.
13. Hosseini, Hamzeh; Mousavi, SeyedEbrahim (2001). Study of mental health status of incoming students of academic year of 2001-2002, Mazandaran University of Medical Sciences; *Journal of Mazandaran University of Medical Sciences*, 10th year, No. 28, pp. 23-32.
14. Hosseinfard, Seyed Mehdi et al. (2005). Epidemiology of mental disorders in students of Rafsanjan; (*Andisheh&Raftar*) *Thinking & Behavior Quarterly Journal*, Year XI, No. 1.

15. Jalalian, Mehrdad (2012). Writing for academic journals: A general approach. *Electronic Physician Journal*. 2012; 4(2): 476-477. Available online at: <http://www.ephysician.ir/2012/476-477.pdf>
16. Javidi, Hojatolah (1994). Epidemiological study of mental disorders in Marvdasht region (Fars province); *Clinical Psychology*, Master's thesis, Psychiatric Institute of Tehran.
17. Kaplan, H.I, Sadock B.J. *Synopsis of psychiatry 8th ed Philadelphia: William, Wilkins company*. 1998.
18. Kessler, R.C., McGonagle, K.A., Zhao, S., Nelson, C.B., Hughes, M., Eshleman, S., Wittchen, H.U., & Kendler, K.S. (1994). Life time and 12 month prevalence of DSM – III – R Psychiatric disorders in the United State. *Archives of General psychiatry*, 51, 9-19.
19. Khosravi, Shamsali (1995). Epidemiological study of mental disorders in urban and rural areas of Borougen, ChaharMahalBakhtiari; *Clinical Psychology*, Master's thesis, Psychiatric Institute of Tehran.
20. Kokabeh, Farrokh (1994). Epidemiological study of mental disorders in rural areas of Azarshahr, Tabriz; *Clinical Psychology*, Master's thesis, Psychiatric Institute of Tehran.
21. Medianos, M. G., Stefanis, C.N., & Madianou, D. (1987). Prevalence of mental disorders and utilization of mental health services in two areas of greater Athens. In B.Cooper, G. Helgason (Eds). *Journal of Psychiatric Epidemiology*, London: Croom Helm (p.p. 86-372).
22. Mohammadi, Antas (2002). Epidemiological study of mental disorders in high school students in the city of ShahinShahr; *Clinical Psychology*, Master's thesis, Psychiatric Institute of Tehran.
23. Mohammadi, MohammadReza; Davidian, Haratoon; Nobala, AhmadAli (2004). Epidemiology of psychiatric disorders in Iran; *Hakim Research Journal*, Volume VI, Issue 1, pp. 50-64.
24. Mousavi, Mohammad; Adli, Mehrdad; Mikoyee, Pouyeh (2000). The prevalence of depression in students aged 15 to 17 of district one high schools in Bandar Abbas in 1999. *Hormozgan Medical Journal*, Year III, No. 2, Summer, 2000, pp. 16-19.
25. Noorbala, Ahmad Ali; Mohammad, Kazem; Bagheri-Yazdi, Seyed Abbas (2000). Review of mental disorders prevalence in Tehran. *Hakim Research Journal*, Volume II, No. 4, pp. 213-223.
26. Omoid, Abdullah; Sazoor, Seyed Ali; Akasheh, Goudarz (2004). Epidemiology of mental disorders in Natanz, Isfahan; (*Andisheh&Raftar*) *Thinking & Behavior Quarterly Journal*, Year VIII, No. 4, pp.32-38.
27. Palahang, Hassan; Nasr Esfahani, Mehdi; Barahani, Mohammad Naghi; ShahMohammadi, Davood (1997). Epidemiological study of mental disorders in Kashan city. (*Andisheh&Raftar*) *Thinking & Behavior Quarterly Journal*, Year II, No. 4, pp. 19-27.
28. Robert, Clifford, adra(1998) prevalence of psychopathology among children and adolescents. *AMJ psychiatry*. 1998 June; 715-725.
29. Roca, M.(1999). Mental disorders on the island of Formentera, Spowin. *Social Psychiatry and Psychiatric Epidemiology*, 34, 420-415.
30. Sadeghi-Movahed, Fariba et al. (2009). Study the effect of coping skills training on mental health status of students; *Journal of Ardebil Medical Sciences*, Volume VIII, Number III, pp. 261-269.
31. Sadock, B.J., & Sadock, A. (2000). *Comprehensive textbook of psychiatry*, Baltimore: Lippincott Williams& Wilkins.
32. Stansfield, S.A., & M.G. (1991). Social class and minor psychiatric disorder in British civil servant: a Validated screening survey using the general health questionnaire. *Psychological Medicine*, 22, 739-449.
33. Sarterbus, Norman (1994). *Mental health programs; Concepts and principles*. Translated by: ShahMohammadi, David; Palahang, Hassan and Yaghobi, Norollah; Tehran; Majd Publication.
34. Weissman, M.M., Mayers, J.K. & Harding, P.S. (1978). Psychiatry disorders in a U.S. Urban Community. *American Journal of psychiatry*, 135, 456-462.
35. WHO (1993). Human right: The vulnerability of mentally ill. *Press Release*, 15,44
36. Yaghobi, Norollah; Nasr Esfahani, Mehdi; Shah Mohammadi, Davood (1996). Epidemiological study of mental disorders in urban and rural areas of Someesaracity (Guilan province). (*Andisheh&Raftar*) *Thinking & Behavior Quarterly Journal*, Year I, No. 4, pp. 55-65.

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## Non Economic Factors Effecting on Farmers Attitudes towards Participatory Irrigation Management (Case Study: Golestan Province, Iran)

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**Abstract:** The present study is the kind of descriptive–correlation and the purpose of this study is to investigate and analyze non economic factors effecting on farmers attitudes towards participatory irrigation management. On the basis of Kochran formula, the numbers of 190 persons are accidentally selected by the using stratified sampling and cluster random sampling and finally 186 questionnaires are analyzed. The farmers as members of Golestan province water users' association made up the statistical population of this research. The tool of research was questionnaire which its reliability approved by the masters and knowledgeable persons and its validity computed 0.77. The data analysis is accomplished by using the SPSS 13 software. The research results shown that there was a significant relation among the term of farmer's membership in water users' association, farm distance to the nearest agricultural service center, level of using the informational resources, level of extensional contacts, social participation and solidarity with Farmer's attitudes towards participatory irrigation of management. The gotten results from stepwise regression shown social solidarity, farm distance to the nearest agricultural service center and the level of using of informational resources forecast 32% of changing the farmer's attitudes toward participatory irrigation of management.

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**Key Words:** Non Economic, Attitude, Farmers, Participatory Irrigation of Management, Golestan Province

### Introduction

Increasing agricultural production in Iran for various reasons including, price stability, the improvement of per capita income sector workers and increasing needs of society to non-oil foreign exchange resources, has become an inevitable necessity and since that not easily has changed obtained the volume of water equivalent to 130 billion cubic meters per year the only way will remain is efficient using of water[22]. Human Development Report 2008 - 2007 UNDP also expressed that until 2080, climate change is capable to increase the number of people around the world face water shortages, to around 8/1 billion[18]. The major challenges' facing the dilemma of the country is water. When there is a water problem, agriculture as an employment industry of the country would be stopped [15]. Experts say the country's water resources management is not appropriate in current circumstances has led to decline in recent years groundwater resources and reducing agricultural acreage in some areas[2]. The agricultural sector due to reasons such as; loss of water supply source to local consumption, high water losses in agricultural fields, inappropriate shape and size of farms with irrigation water, lack of farmers knowledge of making optimum use of water, depletion rapid

infrastructure, low quality and lack of using appropriate irrigation network methods, low irrigation efficiency and irrigation losses are very high[11]. The principles of sustainable development essential for the water industry and should have looked it as a business opportunity not limitation and on the other hand to achieve sustainable development of water needed for a more comprehensive approach that the issue of water management along with institutional mechanisms of participation, according to a new organization will seek [3 and 8]. Therefore, in order to water sustainability, water management overall system should be designed to look like that all farmers are more eager to participate in productivity and production advantage of water resources [9].

According to the pre challenges on water management in Iran are necessary, using appropriate methods of irrigation and improved agricultural water management for increasing agricultural production and improve the environment, making effective use of agricultural water. On the other hand, considering that irrigation system has designed by the government without the participation of farmers, Organization of Water Affairs is facing to heavy costs of operation and maintenance because farmers don't feel responsibility about

it[1]. Thus, participation of farmers through new participatory irrigation management approach is importance in improving agricultural water management[10]. The term participatory irrigation management (PIM) refers to farmers participate in all levels of management including planning, design, construction, operation and maintenance and investment, decision rules and monitoring and evaluation of irrigation systems. On the other hand participatory irrigation management in a manner usually refers to the participation that will increase operational authority and responsibilities farmers in the management process [19 and 14]. Reviewing a literature was clear that some researchers are following implemented particular stage in this study. Shahrodi *et al*[17], Norozi *et al*[13], YaghobiNejad[21], Khoshab and Namazi[12], Aminian and Khayati [1], Chizari and Noroozi[7], Rahman *et al*[16], Damianos and Giannakopoulos[7], Vermillion [20], Ben-Ayed[5] and Bagdi[4].

This research also in line with previous studies in response to this question is designed that if politicians want a part of general or regional water management to farmers should leave what incentives is effective on their attitude towards participatory irrigation management? The present study aimed noneconomic factors affecting on attitude of farmers towards participatory irrigation management has been implemented and it consists of specific objectives:

- 1-Describe the noneconomic characteristics of farmers.
- 2-Relationship between the attitudes of farmers towards participatory irrigation management with noneconomic characteristics.
- 3-Predict changes in attitudes of farmers towards participatory irrigation management based on noneconomic characteristics.

### Methods

The current study is used descriptive and correlative methods. Statistical society of the research includes all farmers that are members of cooperative water users in Golestan (N=11417). Sample size is estimated 190 persons by Cochran formula. Stratified cluster proportion and randomly sampling method is used. As such, desired townships are formed the population of classes. Next, using cluster random sampling with regard to each class to act as a cluster sampling was attempted. As such, a cooperative from each cluster was selected and with using of simple random sampling in cooperative action was collected the desired information. Finally, 186 questionnaires were analyzed. Questionnaire was the most important tool of data collection. In order to confirm the appearance and content validity of the questionnaire several versions of questionnaire gave to extension and agricultural educational professors and a number of experts of

agriculture organization of Tehran province and Golestan province and after several stages revised final approval was obtained. To test reliability, 30 questionnaires completed by farmers and cooperatives members and Cronbach alpha were obtained 0/90. For analysis the statistics of mean, standard deviation, coefficient of variation and linear regression data was used using SPSS version 13.

### Results and Discussion

#### Describe the personal characteristics of farmers

The results showed that the average age of farmers was 45.91 and they have 24.69 years of agricultural activities. The average of duration of farmers' membership in the water users' association was 9.60 years respectively and the average of farm distance to the nearest agricultural services was 55.10 km (Table 1).

Table 1  
Farmers' personal characteristics (n=186)

Variables	Mean	Standard Deviation	Min	Max
-Age (Year)	45.91	11.98	24	81
-Agricultural experience (Year)	24.69	36.13	3	70
-Duration of farmers' membership in the water users' association (Year)	9.60	5.34	1	18
-Distance between farm and agricultural service centers (Km)	10.55	9.70	1	30

#### Describe characteristics determine the educational

To determine farmers used the source of agricultural information 7 source of information are defined and for assessing exposure of agricultural extension to farmers, six items mentioned that farmers answer questions from any to very high. Ranking statistics shows that the most important source of information both local farmers and neighbors, and the least important source of agricultural programs are radio (Table 2). The most important type of extensional contact with extension agent in central services and the least important of this type were studied extension publication (Table 3).

Table 2  
Classification of farmers' using source of information (n=186)

Information sources	Mean*	Standard Deviation	CV	rank
-The local farmers and neighbors	3.41	1.16	34%	1
-Progressive farmers	3.53	1.45	41%	2
-Agricultural TV programs	2.52	1.74	69%	3
-Advised of local leaders	2.34	1.91	81%	4
-Recommended of council place	1.78	1.69	94%	5
-Agricultural radio programs	1.38	1.42	102%	6

\*any=0, Very little=1, little=2, moderate=3, high=4, and very high=5

Table 3  
Classification of various type of farmers' extensional contacts (n=186)

extensional contacts	Mean*	Standard Deviation	CV	rank
-Meeting extension agent at Agricultural Service Center	3.20	1.57	49%	1
-Attending in educational extensional classes	2.81	1.95	69%	2
-Extension agent meet you in the village	2.54	1.79	70%	3
-Representation of extensional films	2.34	1.91	81%	4
-Recommended of Islamic Consultative of village	1.78	1.69	94%	5
-Study extension publications	1.46	1.67	114%	6

\*any=0, Very little=1, little=2, moderate=3, high=4, and very high=5

#### Describe the social characteristics of farmers

For measuring social characteristics were used two components of social solidarity and social participation, for this purpose to measure social solidarity were used the six items and for social participation were used 10 items from any to very high. Table 4 shows the priority of each these indicators. So that the first priority in the social solidarity items is helping other farmers in case of problems and the last priorities allocated to disputes over everyday farmers issues and in social participation variable the first priority was allocated to consultations on everyday problems and the last priorities was allocated to cooperation with the mobilization site.

Table 4  
Classification of social solidarity and social participation among farmers (n=186)

Social Solidarity	Mean*	Standard Deviation	CV	rank
-To help other farmers in case of problems*	4.60	0.83	18%	1
-Mass fighting in the village**	4.53	0.91	20%	2
-To help other farmers in the farm affairs*	4.43	0.91	20%	3
-Participate in celebrations and religious ceremonies in the village*	4.47	1.01	22%	4
-Participation in educational –extensional classes along with other farmers*	4.33	0.99	22%	5
-Disagreements over daily issues with other farmers**	3.62	1.27	35%	6
Social Participation	Mean*	Standard Deviation	CV	rank
-Talk about everyday problems with other farmers*	4.04	1.01	25%	1
-Cooperation with organizations and institutions below:				
Mosque attendance*	4.24	1.22	28%	2
Cooperation with village centers*	3.77	1.21	32%	3
Cooperation with the Agricultural Service Center*	3.72	1.25	33%	4
Collaboration with rural cooperative *	3.63	1.43	39%	5
Cooperation with the Islamic Consultative of village*	3.55	1.41	39%	6
-Negotiate with officials regarding the village problems*	3.70	1.5	40%	7
-Express ideas and opinions while attending in educational classes*	3.63	1.51	41%	8
-Association in cooperation with parents and school teachers*	3.31	1.77	53%	9
-Collaboration with site mobilization*	2.76	1.80	65%	10

\*any=0, Very little=1, little=2, moderate=3, high=4, and very high=5

\*\*any=5, Very little=4, little=3, moderate=2, high=1, and very high=0

**Describe the attitude of farmers towards participatory irrigation management**

To measure attitudes of farmers towards participatory irrigation management was mentioned seven items to respondents' rate their approval or opposition idea and expressed them from completely disagree to completely agree. Increased farmers sense of responsibility in cooperation with each other in water management and reduce disputes concerning problems related to water management, allocated respectively the first and the last priority (Table 5).

Table 5  
Classification of the farmers attitude towards participatory irrigation management (n=186)

Attitudes	Mean*	Standard Deviation	CV	rank
-Cooperation in utilization and maintenance of water causes a sense of responsibility and increase confidence in the farmers*	3.44	0.64	18%	1
-Water resources are a divine blessing that God has bestowed upon us and no need for grouping management and planning**	3.67	0.71	19%	2
-I know everything about agricultural water use and am not need any help from extension agents**	3.52	0.73	20%	3
-Farmers cooperation in using water caused the farmers a sense of independence and ownership of water resources *	3.37	0.76	22%	4

-How to cooperate in using agricultural water makes better use of water resources and reducing costs *	3.43	0.84	24%	5
-Do not intend to use a group of water resources**	3.22	1.09	33%	6
-With farmers cooperation, disputes about water problems is reduced*	2.87	1.31	45%	7

\* Completely disagree=0, disagree=1, no opinion=2, agree=3, completely agree=4  
 \*\* Completely disagree=4, disagree=3, no opinion=2, agree=1, completely agree=0

**Relationship between attitudes of farmers towards participatory irrigation management and noneconomic variables**

correlation coefficients shows that there was a positive and significant correlation between duration of farmers membership in the water users' association, use of informational resources, the rate of extensional contacts, social solidarity and participation with farmers attitudes towards participatory irrigation management at 1 percent level. The relationship between the farm distance to the nearest agricultural services is negative and significant (Table 6).

Table 6  
Correlation levels between attitudes of farmers towards participatory irrigation management and noneconomic variables

Variables	Farmers' attitude toward participatory irrigation management	
	r	p
-Age	-0.057	0.439
-Duration of farmers membership in the water users' association	0.345**	0.000
-Farming experience	0.013	0.861
-Distance between farm and agricultural service centers	-0.044*	0.000
-Using information sources	0.315**	0.000
-Rate of extension	0.285**	0.000

contacts		
-Social participation	0.461**	0.000
- Social solidarity	0.479**	0.000

\*\* P ≤ 0.01

The following studies confirm the present results. Shahroudi *et al* [17] are considered that improving extensional contacts, expanding appropriate channels of information effect in the attitude of farmers in developing and implementing effective agricultural water management practices.

Norouzi *et al* [13] and Chizari and Noroozi [6] achieved positive and significant relationship between extensional contacts, the rate of using communication channel by Wheat farmers with their attitudes about agricultural water management.

Yaghoubi Nejad [21] found a positive and significant relationship between the use of extensional and educational services with increasing attitude of utilizations' towards the water.

Rahman *et al* [16] achieved significant relationship between the ages of farmers and their environmental attitudes.

Damianos and Giannakopoulos [7], Vermillion [20], Ben-Ayed [5] and Khoshab and Namazi [12] believed that there was a significant relationship between solidarity and correlation of rural people and willingness of them to participate in activities.

Bagdi [4] and Amin and Khayati [1] were found that increasing farmers' participation in social activities caused them more favorable view towards the participatory projects in rural places in their study.

#### Determine changes in farmers' attitude towards participatory irrigation management based on noneconomic variables

To determine estimating equation the attitude of farmers towards participatory irrigation management based on independent variables was used multiple regression analysis stepwise method. After entering all significant variables, level of social solidarity, the farm distance to the nearest agricultural services, usage of information sources remained in the equation. Ability of these variables explained 34% (R = 0.347) of changes in farmers' attitude towards participatory management of irrigation.

Table 6

Multiple Linear Regression stepwise Analysis dependent variables: farmers' attitude towards participatory irrigation management

Independent variable	R square	Adjusted R Square	B	Beta	T
Constant	-----	-----	1.587	-----	6.478
Social solidarity (X )	0.229	0.225	0.330	0.361	5.695
Distance between farm and agricultural service centers (X )	0.333	0.325	-0.017	-0.310	-4.835
Using information sources (X )	0.347	0.336	0.079	0.124	1.977

To forecasting estimating equation according to given data and regression analysis to be significant in this study, the following equation can be estimated farmers attitudes towards participatory management of irrigation:

$$Y = 1.587 + 0.330(X) - 0.017(X) + 0.079(X)$$

#### Conclusions and Recommendations

Results of stepwise multiple regression indicated that level of social solidarity, the farm distance to the nearest agricultural services, usage of information sources explained 34% of the variable of changes of the farmers attitude towards participatory irrigation management. Therefore recommended that:

-In order to increase and enhance participation and social solidarity among farmers is proposed that extensional programs be held for adoption advocacy of cooperative activities in the village.

-Explained intermediate representation in some remote areas of agricultural service centers and using more efficient extension agent and experts in order to create favorable perception of water shortage and formally applying modern methods of water management is suggested.

-To promote a favorable attitude of farmers towards participatory irrigation management advocates met extension agent with farmers, offering educational - extension classes necessary to formally cooperate in agricultural water management and use of communication channels such as local farmers and neighbors together and leading farmers and producing Local television and compile the best option in achieving these are important.

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**Resources**

- 1-Amini, A.M. and Khayati, M. (2006). Affecting factors on the failure of established water users' association project (using regression). *Agricultural and Development Economics*, 14(53):63-91.
- 2- Anonymous. (2008). Challenges in water management in the country. (Last review 22 of Khordad 2008). <http://www.iscanews.ir>
- 3- Ashley, R., Blackwood, D., Butler, D., Davies, J., Jowitt, P. and Smith, H. (2003). Sustainable decision making for UK water industry. *Engineering Sustainability*. 156 (1): 41-49.
- 4-Bagdi, G.L. (2005). People's participation in soil and water conservation through watershed approach. First edition. India: International book distributing CO.
- 5-Ben-Ayed, M. (2002). People's participation in a rural development program in Tunisia: A case study -University of Missouri .dissertation .D.Ph .United ,MO ,Columbia
- 6-Chizari, M. and Noroozi, O. (2008). Attitude of Nahavand Township, Iran wheat farmers toward on-farm water management (OFWM). *American-Eurasian J. Agric and Environ. Sic*, 3(2): 233-240.
- 7-Damianos, D. and Giannakopoulos, N. (2002). Farmers' participation in agri-environmental schemes in Greece. *British Food Journal* 104, 261-274
- 8-Guterstam, B. (2008). Toward Sustainable Water Resource Management in Central Asia. Helsinki University of Technology. [On-line] Available at: [www.water.tkk.fi/English/wr/research/global/material/CA\\_chapters/02-CA\\_Waters-Guterstam.pdf](http://www.water.tkk.fi/English/wr/research/global/material/CA_chapters/02-CA_Waters-Guterstam.pdf)
- 9-Heyd, H. and Neef, A. (2004). Participation of local people in water management: evidence from the Mae SA Watershed, Northern Thailand. International Food Policy Research Institute, Washington.
- 10-Heydarian, A. (2003). Transfer Management: Methods, Obstacles and Solutions. Third technical workshop on water users' participation irrigation management network. Tehran: Karaj.
- 11-Keshavarz, A. (2000). Recommendations on policies and programs in Iran and irrigation water. Agricultural extension organization. Tehran.
- 12- Khoshab, A. and Namazi, A. (2006). Study the causes of failure of the farmers welcomed the plans for popular participation. National Conference on Irrigation and Drainage Network Management Chamran University.
- 13- Norozi, A., Chizari, M., Mohseni, A. and Baghayi, M. (2006). Cultural factors, social attitudes affecting on Wheat farmers of Nahavand about agricultural water management. The 1th Regional Conference Exploitation of Water Resources of Karon and Zayanderod (Opportunities and challenges).
- 14- Peter, J. R. (2004). Participatory irrigation management. International network on participatory irrigation management, Washington DC. INWEPF/SY/2004 (06). [Online] Available at: <http://www.maff.go.jp/inwepf/document/inaugural/inpim-note.pdf>.
- 15-Rahgozar.(2001). Water need crisis management. Khorasan magazine. 2001 Mordad.
- 16-Rahman, M.Z., Mikuni, H. and Rahman, M.M. (1999). Towards sustainable farming development: The attitude of farmers in a selected area of Shimane prefecture, Japan. *Journal of Sustainable Agricultural*, 14(4), 19-33.
- 17-Shahrodi, A., Chizari, M. and Pezeshki rad, Gh. (2008). Impact water users' association on farmers' attitude towards agricultural water management. Case study on Khorasan province. *Journal of Agricultural Economics and Development (Agricultural Science and Technology)*, 22 (2):71-85.
- 18- UNDP. (2007). Human Development Report 2007/2008. Fighting climate change: Human solidarity in a divided world. [On-line] Available at: <http://hrd.undp.org>
- 19- Vermillion. D.L. (1997). Impact of Irrigation Management Transfer. Research Report 11. Colombo, Sri Lanka: IIMI.
- 20-Vermillion, D. L. (2000). Guide to monitoring and evaluation of irrigation management transfer. The Japanese institute for irrigation and drainage and international network on participatory irrigation management.
- 21- YaghobiNejad, M.(2003). How to promote and realize the possibility and efficiency of water reform participatory water users' management Proceedings of Eleventh Conference of the Iranian National Committee on Irrigation and Drainage.
- 22- Zibaye, M. (2003). Determine the efficiency of investment in irrigation systems installed in Fars province. Conference Proceedings Agricultural Financing, Experiences and Lessons. TarbiyatModdares University. Economics Research Institute.

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## Antioxidant Role of both Propolis and Ginseng against Neurotoxicity of Chlorpyrifos and Profenofos in Male Rats

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**Abstract:** The present study was an attempt to evaluate the toxic effect of both Chlorpyrifos and profenofos (organophosphorous insecticides) each alone and in their combinations with either propolis or ginseng and as well known that propolis and ginseng have been reported to be effective antioxidant, therefore, the present study was aimed to elucidate the possible ameliorative role of propolis and ginseng in alleviating the toxicity of both Chlorpyrifos and profenofos when given to male rats. This was done through studying the effects of both Chlorpyrifos and profenofos on some antioxidant enzymes in liver, Kidney and brain homogenates and by measuring acetylcholinesterase as well as histopathological changes in vital organ like Brain. Animals were divided into nine groups; The 1<sup>st</sup> (Control group): Animals received 1ml of distilled water orally daily for 8 weeks, The 2<sup>nd</sup> (Chlorpyrifos treated group) Animals were daily received oral doses of Chlorpyrifos (6.75 mg/Kg b.wt.) for 60 days, The 3<sup>rd</sup> (Profenofos treated group) Animals were received orally Profenofos (20 mg/Kg b.wt.) daily for 8 weeks, The 4<sup>th</sup> (Propolis treated group) Animals were received orally Propolis extract (70mg/kg) daily for 8 weeks, The 5<sup>th</sup> (Ginseng treated group) Animals were given orally Ginseng extract (200mg/Kg b.wt.) for 8 weeks daily, The 6<sup>th</sup> (Chlorpyrifos + Propolis treated group) Animals were given orally Chlorpyrifos (6.75 mg/Kg) and then co-administered with Propolis extract (70mg/kg b.wt.) for 8 weeks daily, The 7<sup>th</sup> (Chlorpyrifos+Ginseng treated group) Animals were given orally Chlorpyrifos (6.75 mg/Kg b.wt.) and then co-administered with Ginseng extract (200mg/Kg b.wt.) for 8 weeks daily, The 8<sup>th</sup> (Profenofos +Propolis treated group) Animals were given orally Profenofos (20 mg/Kg b.wt.) and then co-administered with Propolis extract (70mg/kg b.wt.) for 8 weeks daily, The 9<sup>th</sup> (Profenofos +Ginseng treated group) Animals were given orally Profenofos (20 mg/Kg) and then co-administered with Ginseng extract (200mg/Kg) as mentioned above for 8 weeks daily. Results showed that there was a correlation between CPF and PRF administration and the highly significant increase of the antioxidant enzymes, Cortisol and neurotransmitter (Acetylcholinesterase). In contrary to these actions, co-administration of propolis and ginseng to CPF and PRF-treated rats retrieved almost most of these biochemical parameters to normal levels. On the other hand, CPF and PRF showed histopathological alterations in brain of male rats like hemorrhage and mild degeneration, while administration of both propolis and ginseng highly ameliorate these dangerous neurotoxicity markers.

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**Keywords:** Chlorpyrifos, Profenofos, Propolis, Ginseng, neurotoxicity, antioxidant enzymes, Acetylcholinesterase.

**Abbreviations:** CPF, Chlorpyrifos; PRF, Profenofos; MDA, Malondialdehyde enzyme; SOD, Superoxide dismutase; CAT, Catalase; NO; Nitric oxide, GPX; Glutathione peroxidase, GSH; Glutathione reduced, G-6-Ph; Glucose-6-phosphate

### 1. Introduction

Exposure to pesticides may involve large segments of population which include agriculture workers and their families, besides the general population who may be exposed through home application of pesticides or via residues on food [1]. There is an urgent need for the use of pesticides to protect economic plants and animals against pests infesting them. Several organophosphorus insecticides are widely used in various purposes. The unavoidable increased use of many new pesticides may cause great hazards to the living organisms, carelessly or wrongly application lead to pollution for the total ecosystem (i.e., air, earth, plant, water, animal and human

ecosystem). A considerable numbers of these pesticides were reported to have acute, chronic, histopathologic and teratogenic activities [2].

Chlorpyrifos (CPF) is an effective organophosphate; (OP) pesticide used heavily throughout the world for agriculture and domestic purposes. The main target of OP pesticides is acetylcholinesterase (AChE), which hydrolyse acetylcholine (ACh) in cholinergic synapses and at neuromuscular junctions [3] this results in the accumulation of ACh in the synapses which in turn induces hyperactivity in cholinergic pathways. Besides, CPF elicits a number of other effects including hepatic dysfunction, immunological abnormalities,

embryotoxicity, genotoxicity, teratogenicity, neurochemical, and neurobehavioral changes [4].

The organophosphorus (OP) insecticide Profenofos (o-4-bromo -2- chlorophenyl-O- ethyl S-propyl) phosphorothioate is used heavily in cotton-growing areas of wide area of the world such as; Eastern Australia, Northern Africa and various areas of America [5]. Besides Profenofos is a broad-spectrum organophosphate insecticide and acaricide used widely used for agricultural and household purposes [6]. Profenofos caused different symptoms of toxicity and revealed some biochemical changes especially in the enzymes activity of the liver and brain following two sublethal doses of profenofos in mice [7].

To control the level of reactive oxygen species (ROS) and to protect cells under stress conditions, mammalian tissues contain several enzymes scavenging ROS such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX) and glutathione S-transferase (GST), and reduced glutathione (GSH) Some compounds also contribute to detoxification process from ROS such as propolis [8].

Natural products are a promising source for the discovery of new pharmaceuticals. In the last decades, several works dealing with propolis' composition and biological properties have been published, revealing the interest of researchers on this bee product and its potential for the development of new drugs [9].

Propolis is a resinous hive product collected by honeybees from plants, showing a very complex chemical composition [10]. It has been used in folk medicine since ancient times, due to its many biological properties, such as antibacterial [11], antitumor [12], and immunomodulatory [13].

Propolis also contains more than 300 biochemical constituents, including mostly a mixture of polyphenols, flavonoid aglycones, phenolic acid and their esters, and phenolic aldehydes and ketones, terpenes, sterols, vitamins, amino acids [14].

Ginseng is a well-known medicinal herb in traditional Asian medicine and is considered an adaptogen. *Panax ginseng* C.A. Meyer (Araliaceae), which grows in China and Korea, has a variety of beneficial biological actions that include anti-carcinogenic, anti-diabetic-inflammatory effects, as well as cardiovascular protection and neuroprotection [15].

## 2. Materials and Methods

### 2.1. Test insecticide

**2.1.1 Chlorpyrifos** was produced by Misr for Agricultural Development Company, Cairo, Egypt. Under trade name Dursban and was stored at 4°C until stock solution preparation. The insecticide (CPF) was orally administered at a dose level equivalent to 1/20 LD<sub>50</sub> (6.75 mg/kg b.wt.) in distilled water for 60 successive days, this selected dose of the insecticide was based on previous studies in which 1/20 LD<sub>50</sub> of

CPF induced biochemical alterations in rats without morbidity [16]. Stock solution was prepared by bringing Chlorpyrifos to room temperature then taking a certain amount by pipette from the Chlorpyrifos bottle and dilute it in distilled water (**0.25 ml of Chlorpyrifos was dissolved in 250 ml dist. water**) and diluting it in tween 80 to ensure rapid and complete absorption and we prepare 250 ml only to prepare the working solution freshly for each day of dosing [17, 18].

**2.1.2 Profenofos** is a pale yellow liquid; it was produced by Ciba-Geigy, Pharmacological Company, Scientific office Cairo, Egypt. under trade name: Selecron 72% EC, Profenofos was given at a dose of (20mg/Kg b.wt.) which represent 1/10 LD<sub>50</sub>, where the LD<sub>50</sub> value of Profenofos is (200 mg/Kg) according to Weil [19] and this selected dose of the insecticide was based on Weil studies in which 1/10 LD<sub>50</sub> of Profenofos induced biochemical alterations in rats without morbidity. Tap water was used for preparing emulsion of Profenofos immediately before use, Stock solution was prepared by bringing Profenofos to room temperature then taking a certain amount by pipette from the Profenofos bottle and diluting it in distilled water (1.97 ml of Profenofos was diluted in 250 ml dist. water) we prepare 250 ml only of working solution freshly for each day of dosing [20].

### 2.2. Extracts

#### 2.2.1 Propolis extract preparation:

In this study, Propolis powder extract (70% ethanolic extract) was obtained from (Dosis IMP & EXP. Co, Ltd) China. Propolis was dissolved in dist. water and administered orally for 60 successive days via gastric tube at dose 70 mg/ Kg [21,22]

#### 2.2.2 Ginseng extracts preparation:

Red Ginseng powder (Supplied by Tsumura Pharmaceutical Co., Tokyo, Japan) was administered orally at dose (200 mg/Kg b.wt.) [23] for 60 successive days via a gastric tube. The Ginseng extract was suspended in tap water just before use and the dose was calculated according to the animal's body weight on the week before using.

### 2.3. Animals

The present study was carried out at Zoology Department, Faculty of Science - Zagazig University, using (one hundred and ten) (110) clinically healthy mature adult male albino rats. The animals were obtained from the Animal House of Faculty of Veterinary Medicine, Zagazig University, Their weights ranged from (200-250gm) each. The animals were housed in standard conditions, where the animals were housed in metal cages and bedded with wood shavings and kept under standard laboratory conditions of aeration and room temperature at about 25°C. The animals were allowed to free access of standard diet and water *ad libitum*. The animals were accommodated



to the laboratory conditions for two weeks before being experimented.

#### 2.4. Experimental design

After the period of acclimation, animals were divided into nine groups with 10 animals in each as :

**I) The 1<sup>st</sup> (Control group):** Animals received 1ml of distilled water orally daily for 8 weeks.

**II) The 2<sup>nd</sup> (Chlorpyrifos treated group):** Animals were daily received oral doses of Chlorpyrifos (6.75 mg/Kg) for 8 weeks using metallic stomach tube.

**III) The 3<sup>rd</sup> (Profenofos treated group):** Animals were received orally Profenofos (20 mg/Kg) daily for 8 weeks using metallic stomach tube.

**IV) The 4<sup>th</sup> (Propolis treated group):** Animals were received orally *Propolis* extract (70mg/kg) daily for 8 weeks using metallic stomach tube.

**V) The 5<sup>th</sup> (Ginseng treated group):** Animals were given orally Ginseng extract (200mg/Kg) for 8 weeks daily using metallic stomach tube.

**VI) The 6<sup>th</sup> (Chlorpyrifos + Propolis treated group):** Animals were given orally Chlorpyrifos (6.75 mg/Kg) and then co-administered with *Propolis* extract (70mg/kg) for 8 weeks daily.

**VII) The 7<sup>th</sup> (Chlorpyrifos+Ginseng treated group):** Animals were given orally Chlorpyrifos (6.75 mg/Kg) and then co-administered with *Ginseng* extract (200mg/Kg) for 8 weeks daily.

**VIII) The 8<sup>th</sup> (Profenofos +Propolis treated group):** Animals were given orally Profenofos (20 mg/Kg) and then co-administered with *Propolis* extract (70mg/kg) for 8 weeks daily.

**XI) The 9<sup>th</sup> (Profenofos +Ginseng treated group):** Animals were given orally Profenofos (20 mg/Kg) and then co-administered with *Ginseng* extract (200mg/Kg) as mentioned above for 8 weeks daily.

#### 2.5 Biochemical Assays

Blood samples were collected after the end of the experiment from the retro-orbital vein, which is a simple, convenient and successful procedure that allows bleeding of the same animal more than one time with minimal stress [24]. After the last administration of the drug at the end of 8<sup>th</sup> week, individual blood samples were drawn by orbital puncture (from eye plexus) using microhematocrit capillary tubes (Lancer, Athy, County-Kildare, Republic of Ireland), Serum was harvested from blood without EDTA and then blood samples were transferred into Eppendorf tubes and subsequently used for the determination of the following biochemical parameters, The biochemical measurements were performed according to the details given in the kit's instructions.

##### 2.5.1 Determination of serum Cortisol concentration:

Serum cortisol was determined using by biodiagnostic kit method (Biodiagnostic Company, Dokki, Giza, Egypt). The electro chemiluminescence's

immunoassay (ECLIA) is intended for immunoassay analyzer of cobas according to Arakawa et al.[25].

##### 2.6 Preparation of Tissue Homogenate for antioxidant enzymes:

The remainder tissues of liver were used for the analyses of oxidative stress parameters. They were washed with saline and distal water for the removal of blood, and later the fatty parts were removed and blotted over a piece of filter paper. Prior to dissection, tissue was perfused with a 50 mM (sodium phosphate buffer saline (100 mM Na<sub>2</sub>HPO<sub>4</sub> / NaH<sub>2</sub>PO<sub>4</sub>) (PH 7.4) in an Ice containing medium containing 0.16 mg / ml heparin or containing 0.1 mM ethylene di amine tetra acetic acid (EDTA) to remove any red blood cells and clots. Then tissues were homogenized in 5 – 10 ml cold buffer per gram tissue and Centrifuged at 5000 r.p.m for ½ hour. The resulting supernatant was transferred into Eppendorf tubes, and preserved at -80°C in a deep freezer until used for various biochemical Assays [26].

##### 2.6.2 Determination of Catalase activity:

Catalase (CAT) activity was determined by biodiagnostic kit method (Biodiagnostic Company, Dokki, Giza, Egypt), according to the method of Aebi [27].

##### 2.6.3 Determination of Superoxide dismutase activity:

Superoxide dismutase (SOD) activity was determined by biodiagnostic kit (Biodiagnostic Company, Dokki, Giza, Egypt), according to the method of Nishikimi et al.[28].

##### 2.6.4 Determination of Reduced Glutathione (GSH) activity:

Glutathione reduced (GSH) activity was determined by biodiagnostic kit (Biodiagnostic Company, Dokki, Giza, Egypt), according to the method of Beutler et al., [29].

##### 2.6.5 Determination of Glutathione Peroxidase (GPX) activity:

Glutathione peroxidase activity was determined using biodiagnostic kit (Biodiagnostic Company, Dokki, Giza, Egypt), according to the method of Paglia and Valentine [30].

##### 2.6.6 Determination of Lipid peroxide (Malondialdehyde) activity:

Malondialdehyde (MDA) was determined by using Biodiagnostic kit (Biodiagnostic Company, Dokki, Giza, Egypt), according to the method of [31].

##### 2.6.7 Determination of Serum Nitric Oxide (NO):

Nitric oxide (NO) level was determined by using Biodiagnostic kit method (Biodiagnostic Company, Dokki, Giza, Egypt), according to the method of Montgomery and Dymock [32].

##### 2.6.8 Determination of Glucose -6-Phosphate Dehydrogenase:

The enzyme activity is determined by measurement of the rate absorbance change at 340 nm due to the reduction of NADP<sup>+</sup> by using Biodiagnostic

kit method (Biodiagnostic Company, Dokki, Giza, Egypt), according to the method of Kornberg [33].

### 2.7 Estimation of brain neurotransmitters:

#### (Estimation of Dopamine, Epinephrine, nor epinephrine and serotonin content)

Dopamine as well as catecholamines (Epinephrine, nor epinephrine and dopamine) was determined in rat's brain according to the method of Ciarlone [34].

### 2.8 Estimation of acetylcholinesterase:

Acetylcholinesterase was determined by biodiagnostic kit (Biodiagnostic Company, Dokki, Giza, Egypt), according to the method of Ellman et al [35].

### 2.9 Preparation of tissues for histopathological examination

After 8 weeks post drug administration, animals were sacrificed and samples from heart, liver, brain, kidney and testis were fixed in 10% formalin for histopathological studies. Parts of liver were transferred into 10% buffered formalin for histopathological examination, and the remainder tissue was used for the analysis of oxidative stress parameters. Tissue samples were taken from the liver of the necropsied animals and fixed in 10% formalin saline. The trimmed tissues were first washed with tap water followed by dehydration through a graded alcohol series and then passed through xylol and paraffin series before finally blocked in paraffin. The paraffin blocks were cut into 5-6  $\mu\text{m}$  sections using a microtome stained using hematoxylin and eosin and examined under a light microscope [36].

### 2.10 Statistical analysis

Data were collected, arranged and reported as mean  $\pm$  standard error of mean (S.E.M) of nine groups (Each group was considered as one experimental unit), summarized and then analyzed using the computer program SPSS/ version 15.0) The statistical method was one way analyzes of variance ANOVA test (F-test), and if significant differences between means were found, Duncan's multiple range test (Whose significant level was defined as ( $P < 0.05$ ) was used according to Snedecor, and Cochran, [37] to estimate the effect of different treated groups.

## 3. Results

### 3.1 Morbidity and mortality:

Male rats orally administered Chlorpyrifos, profenofos in doses of (6.75 mg/kg) and (200 mg/Kg) respectively for 60 days have shown signs of toxicity (Diarrhea, myosis, increased urination, diaphoresis, nose and eye bleeding and salivation) and no deaths were recorded throughout the experimental groups.

### 3.2 Effect on serum Cortisol:-

It was clearly evident from (Table 1) and (Fig. 1) that the administration of Chlorpyrifos and/or Profenofos each alone in their recommended doses daily for successive 60 days afforded a highly

significant increase ( $P < 0.05$ ) in serum cortisol level after the end of the experiment when compared with control group. Concerning the effect of either Propolis or Ginseng, the same table and figure revealed that Propolis and/or Ginseng treated groups showed non significant changes in serum cortisol level when compared with control group. A non significant increase in serum cortisol level was also recorded in response to treatment of male rats with combinations of either Chlorpyrifos or Profenofos with either Propolis or ginseng compared with normal control group.

### 3.3 Effect on Antioxidant enzymes:

#### 3.3.1 Effect on Catalase:

Regarding the effect of profenofos and Chlorpyrifos on catalase activity of normal rats, Chlorpyrifos and profenofos afforded a marked decrease ( $P < 0.05$ ) in liver homogenates catalase after the end of the study when compared with control group, whereas, Treatment of normal rats with either propolis or Ginseng alone exhibited non significant changes in Catalase of liver after the end of the experiment when compared with control group (Table 2) and (Figs. 2,3) While combinations of Chlorpyrifos, Profenofos with either Propolis or ginseng exhibited a significant decrease in Catalase activity of liver after the end of the study compared with normal control group.

#### 3.3.2 Effect on Superoxide dismutase (SOD):

The results of the study revealed that treatment of normal rats with either of Chlorpyrifos and/or profenofos elicited a highly significant decrease ( $P < 0.05$ ) in liver SOD level after the end of the study when compared with control group. Treatment of normal rats with either propolis or ginseng for 8 weeks elicited a significant increase in SOD activity of the liver after the end of the study. Whereas, the combinations of the plant extracts with the test insecticides afforded non significant changes in the SOD activity of the liver compared with normal control group. Table (3) and Figs. (4,5).

#### 3.3.3 Effect on Malondialdehyde (MDA):

The MDA content of the liver was significantly elevated ( $P < 0.05$ ) in response to treatment of normal male rats with either Chlorpyrifos and/or profenofos for 8 weeks compared with normal control group. The same previous response was reported with propolis, ginseng and their combinations with either Chlorpyrifos or profenofos compared with control group (Table 4) and (Figs. 6,7).

#### 3.3.4 Effect on Glutathione reduced:

It was apparent from (Table 5) and (Figs. 8,9) that treatment of rats with Chlorpyrifos, Profenofos each alone afforded a significant decrease ( $P < 0.05$ ) in liver reduced glutathione after the end of the study when compared with normal control group. On the other hand, the results revealed that Ginseng and/or Propolis induced a non significant change in reduced

Glutathione content of the liver compared with control group.

### 3.3.5 Effect on Glutathione Peroxidase:

The plasma Glutathione peroxidase level was significantly reduced ( $P<0.05$ ) in all groups treated with Chlorpyrifos, Profenofos each alone, propolis, ginseng and their combinations for successive 60 days when compared with normal control group. Whereas, a non significant change to slight decrease was reported in response to treatments with all combinations used except combination of Chlorpyrifos with propolis which showed a significant decrease compared with normal control group (Table 6) and (Figs.10,11).

### 3.3.6 Effect on serum Nitric oxide (No) & Glucose-6-Phosphate Dehydrogenase (G6PH):

The serum Nitric oxide and glucose – phosphate dehydrogenase levels were significantly elevated ( $P<0.05$ ) in groups treated with Chlorpyrifos and /or Profenofos each alone .Whereas, Treatment of normal rats with Ginseng, Propolis and their combinations with both Chlorpyrifos and Profenofos afforded non significant changes ( $P<0.05$ ) after the end of the study when compared with normal control group (Table 7) (Fig.12).

### 3.4 Effect on Acetylcholinesterase:

It was clear from (Table 8) and (Fig.13) that the administration of either Chlorpyrifos or Profenofos each alone to normal rats for successive 60 days afforded a marked decrease ( $P<0.05$ ) in serum acetylcholinesterase activity after two months post administration. Whereas, a non significant change in serum Acetylcholinesterase activity was observed in the groups treated with either Propolis or Ginseng. At the meantime , a significant decrease ( $P<0.05$ ) was recorded in the groups treated with the combinations of the insecticides used with either Propolis or ginseng after 8 weeks post administration except the combination of Profenofos with Propolis which showed a non significant change with control group . Yet the results of the drugs combinations were much higher than that produced with the insecticide alone indicating a good ameliorating effect with the used plant extracts.

#### 3.4.1 Effect on brain Dopamine:

Treatment of normal rats with either Chlorpyrifos or Profenofos for 60 successive days in their recommended doses elicited a marked decrease ( $P<0.05$ ) in plasma dopamine level after 8 weeks post administration when compared with normal control group. Propolis and Ginseng treated groups showed non significant changes in plasma dopamine activity after two months of administration when compared with normal control group. yet their combinations with either Chlorpyrifos or profenofos elicited a significant increase in plasma dopamine concentration when they were compared with each insecticide alone reverting

their values to nearly control values (Table 9) & (Fig. 14).

#### 3.4.2 Effect on brain Serotonin:

The administration of Chlorpyrifos and/or Profenofos in their recommended doses for successive 60 days into normal rats elicited a significant decrease ( $P<0.05$ ) in plasma serotonin level compared to normal control group. Whereas a significant increase ( $P<0.05$ ) was observed in the groups treated with either Propolis or Ginseng alone. Whereas, the combinations of either propolis or ginseng with either Chlorpyrifos or profenofos afforded non significant changes when compared with normal control group, indicating that they reverted the serotonin level to nearly it's normal control level (Table 9) and (Fig 14).

#### 3.4.3 Effect on brain Epinephrine:

Concerning the effect of Chlorpyrifos and Profenofos on serum epinephrine, Chlorpyrifos and/or Profenofos each alone afforded a significant decrease ( $P<0.05$ ) in serum epinephrine level when compared with normal control group after 8 weeks of the insecticides administration. Meanwhile, treatment with either Propolis or Ginseng and their combinations with either chlorpyrifos or profenofos revealed non significant changes after 8th weeks when compared with normal control group (Table 9) and (Fig. 14).

#### 3.4.4 Effect on brain nor-Epinephrine:

The administration of either Chlorpyrifos and/or Profenofos each alone in their recommended doses for successive 60 days into normal rats elicited a significant decrease ( $P<0.05$ ) in neither plasma nor epinephrine content when compared with normal control group. Meanwhile, non significant changes were reported in groups treated with either Propolis or Ginseng each alone and their combinations with either Chlorpyrifos and/or Profenofos after the end of the experiment when compared with normal control group (Table 9) and (Fig. 14). However, these values were significantly elevated ( $P<0.05$ ) when compared with the groups given the insecticides used alone, indicating a potent ameliorative effect of the test plant extracts.

### 3.5 Histopathology:

#### (Group 1): Control group

**The Brain:** Microscopically: normal gyri and sulci of brain tissue (Fig. 15). Normal brain tissue formed of round and pyramidal shaped neurons surrounded by eosinophilic glial fibers.

#### (Group 2): Chlorpyrifos treated group

**The Brain:** Showed fragments of brain tissue separated by large areas of haemorrhage (Fig. 16). Also seen in (Fig. 17) atrophic brain tissue showing few atrophic neurons with pyknotic nuclei and surrounded by excessive glial tissue.

#### (Group 3): Profenofos treated group

**The Brain:** Dilated congested vascular space filled with red blood cells and compressing brain tissue (Fig. 18).

**(Group 4): Propolis treated group**

**The Brain :**The brain of Propolis treated group showing normal gyri and sulci of brain tissue, (Fig. 19).

**(Group 5): Ginseng treated group**

**The Brain :**Normal brain tissue formed of round and pyramidal shaped neurons surrounded by eosinophilic glial fibers (Fig. 20).

**(Group 6): Chlorpyrifos + Propolis treated group**

**The Brain:** The brain of this group showed dilated mild congested vascular space filled with few red blood cells (Fig. 21).

**(Group 7): Chlorpyrifos + Ginseng treated group**

**The Brain:** Photomicrograph of brain tissue showing normal appearance of sulci of brain tissues with very mild haemorrhage in between the compartments of brain tissues (Fig. 22).

**(Group 8): Profenofos+Propolis treated group**

**The Brain:** Brain tissue showing mild congestion with mild haemorrhage in the area between the compartments of brain tissues (Fig. 23).

**(Group 9): Profenofos + Ginseng treated group**

**The Brain:** Photomicrograph of normal brain tissue fragment separated by very mild congested area (Fig. 24).

Table (1): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Cortisol in male albino rats (mean  $\pm$  SE). (N = 7).

	Cortisol ( $\mu$ g/dL)
Control group	1.90 $\pm$ 0.23 <sup>bc</sup>
Chlorpyrifos	7.85 $\pm$ 0.64 <sup>a</sup>
Profenofos	7.54 $\pm$ 0.89 <sup>a</sup>
Propolis	1.81 $\pm$ 0.36 <sup>bc</sup>
Ginseng	1.81 $\pm$ 0.17 <sup>bc</sup>
Chlorpyrifos + Propolis	2.98 $\pm$ 0.72 <sup>b</sup>
Chlorpyrifos + Ginseng	2.81 $\pm$ 0.51 <sup>b</sup>
Profenofos+ Propolis	2.96 $\pm$ 0.39 <sup>b</sup>
Profenofos+Ginseng	2.86 $\pm$ 0.58 <sup>b</sup>

Means within the same column in each category carrying different litters are significant at ( $P \leq 0.05$ ) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

Table (2): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Catalase in male albino rats (mean  $\pm$  SE). (N = 7).

Groups	Antioxidant defense system			
	plasma Catalase (U/ml)	Liver Catalase (U/g)	Brain Catalase (U/g)	Kidney Catalase (U/g)
Control group	159.92 $\pm$ 2.21 <sup>bc</sup>	4.19 $\pm$ 0.01 <sup>a</sup>	8.18 $\pm$ 0.01 <sup>c</sup>	5.49 $\pm$ 0.05 <sup>b</sup>
Chlorpyrifos	77.60 $\pm$ 7.16 <sup>d</sup>	0.50 $\pm$ 0.09 <sup>c</sup>	2.57 $\pm$ 0.02 <sup>f</sup>	12.96 $\pm$ 0.05 <sup>a</sup>
Profenofos	64.87 $\pm$ 17.73 <sup>d</sup>	0.41 $\pm$ 0.13 <sup>c</sup>	2.52 $\pm$ 0.01 <sup>f</sup>	12.91 $\pm$ 0.05 <sup>a</sup>
Propolis	155.07 $\pm$ 0.99 <sup>c</sup>	4.33 $\pm$ 0.03 <sup>a</sup>	8.23 $\pm$ 0.01 <sup>b</sup>	4.61 $\pm$ 0.01 <sup>c</sup>
Ginseng	158.53 $\pm$ 1.08 <sup>c</sup>	4.43 $\pm$ 0.04 <sup>a</sup>	9.24 $\pm$ 0.03 <sup>a</sup>	4.68 $\pm$ 0.07 <sup>c</sup>
Chlorpyrifos +Propolis	163.93 $\pm$ 1.45 <sup>b</sup>	3.72 $\pm$ 0.03 <sup>b</sup>	7.42 $\pm$ 0.02 <sup>d</sup>	4.36 $\pm$ 0.07 <sup>d</sup>
Chlorpyrifos +Ginseng	167.37 $\pm$ 2.47 <sup>ab</sup>	3.57 $\pm$ 0.04 <sup>b</sup>	6.45 $\pm$ 0.02 <sup>c</sup>	4.41 $\pm$ 0.04 <sup>d</sup>
Profenofos + Propolis	175.75 $\pm$ 2.25 <sup>a</sup>	3.72 $\pm$ 0.05 <sup>b</sup>	7.37 $\pm$ 0.04 <sup>d</sup>	4.77 $\pm$ 0.07 <sup>c</sup>
Profenofos + Ginseng	179.90 $\pm$ 3.84 <sup>a</sup>	3.69 $\pm$ 0.02 <sup>b</sup>	7.41 $\pm$ 0.01 <sup>d</sup>	4.74 $\pm$ 0.08 <sup>c</sup>

Means within the same column in each category carrying different litters are significant at ( $P \leq 0.05$ ) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

Table (3): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Super oxide dismutase (SOD) in male albino rats (mean  $\pm$  SE). (N = 7).

Groups	Antioxidant defense system			
	Plasma SOD (U/ml)	Liver SOD (U/g)	Brain SOD (U/g)	Kidney SOD (U/g)
Control group	108.06±3.70 <sup>b</sup>	83.32±0.87 <sup>c</sup>	105.13±0.17 <sup>c</sup>	102.41±0.79 <sup>i</sup>
Chlorpyrifos	22.91±3.77 <sup>d</sup>	20.61±1.52 <sup>de</sup>	194.03±0.23 <sup>b</sup>	281.59±0.22 <sup>a</sup>
Profenofos	20.22±4.02 <sup>d</sup>	24.14±2.98 <sup>e</sup>	196.75±0.15 <sup>a</sup>	277.76±0.51 <sup>b</sup>
Propolis	100.07±2.26 <sup>b</sup>	98.82±0.91 <sup>a</sup>	100.93±0.21 <sup>e</sup>	110.66±0.18 <sup>h</sup>
Ginseng	120.80±2.03 <sup>a</sup>	92.94±1.66 <sup>b</sup>	102.35±0.36 <sup>d</sup>	112.62±0.22 <sup>g</sup>
Chlorpyrifos +Propolis	81.27±1.90 <sup>d</sup>	80.92±1.59 <sup>c</sup>	99.07±0.23 <sup>e</sup>	147.05±0.24 <sup>c</sup>
Chlorpyrifos +Ginseng	92.90±2.20 <sup>c</sup>	79.91±0.88 <sup>c</sup>	95.25±0.09 <sup>f</sup>	140.25±0.03 <sup>d</sup>
Profenofos + Propolis	95.80±3.92 <sup>b</sup>	81.18±1.68 <sup>c</sup>	100.40±0.22 <sup>e</sup>	130.73±0.16 <sup>c</sup>
Profenofos + Ginseng	99.77±10.79 <sup>b</sup>	82.99±1.10 <sup>c</sup>	104.64±0.37 <sup>c</sup>	125.70±0.21 <sup>f</sup>

Means within the same column in each category carrying different litters are significant at ( $P \leq 0.05$ ) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

Table (4): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Malondialdehyde (MDA) in male albino rats (mean ± SE). (N = 7).

Groups	Oxidative stress markers			
	Plasma MDA (nmol/ml)	Liver MDA (nmol/g)	Brain MDA (nmol/g)	Kidney MDA (nmol/g)
Control group	16.50±0.63 <sup>h</sup>	1.74±0.10 <sup>d</sup>	10.39±0.05 <sup>g</sup>	13.27±0.02 <sup>h</sup>
Chlorpyrifos	55.47±1.60 <sup>a</sup>	8.35±0.26 <sup>a</sup>	19.53±0.12 <sup>a</sup>	23.68±0.42 <sup>b</sup>
Profenofos	46.30±1.64 <sup>b</sup>	8.50±0.66 <sup>a</sup>	18.51±0.09 <sup>b</sup>	26.60±0.18 <sup>a</sup>
Propolis	18.04±0.45 <sup>G</sup>	2.81±0.12 <sup>c</sup>	14.44±0.07 <sup>f</sup>	14.42±0.01 <sup>g</sup>
Ginseng	18.39±0.16 <sup>G</sup>	3.30±0.25 <sup>c</sup>	15.15±0.02 <sup>e</sup>	14.79±0.03 <sup>g</sup>
Chlorpyrifos +Propolis	25.52±0.67 <sup>de</sup>	5.38±0.38 <sup>b</sup>	17.34±0.10 <sup>c</sup>	19.27±0.15 <sup>d</sup>
Chlorpyrifos +Ginseng	22.18±0.56 <sup>f</sup>	5.04±0.26 <sup>b</sup>	17.43±0.16 <sup>c</sup>	18.59±0.05 <sup>e</sup>
Profenofos + Propolis	31.69±1.18 <sup>c</sup>	3.66±0.26 <sup>c</sup>	16.25±0.07 <sup>d</sup>	20.10±0.08 <sup>c</sup>
Profenofos + Ginseng	27.74±0.61 <sup>d</sup>	5.05±0.06 <sup>b</sup>	17.32±0.14 <sup>c</sup>	17.75±0.11 <sup>f</sup>

Means within the same column in each category carrying different litters are significant at ( $P \leq 0.05$ ) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

Table (5): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Glutathione reduced in male albino rats (mean ± SE). (N = 7).

Groups	Oxidative stress markers			
	Serum Glutathione reduced (U/L)	Liver Glutathione reduced (U/g)	Brain Glutathione reduced (U/g)	Kidney Glutathione reduced (U/g)
Control group	15.60±0.66 <sup>c</sup>	26.11±0.75 <sup>a</sup>	72.38±0.13 <sup>c</sup>	96.41±0.50 <sup>a</sup>
Chlorpyrifos	8.62±0.47 <sup>f</sup>	6.12±0.48 <sup>c</sup>	37.84±0.14 <sup>G</sup>	44.62±0.30 <sup>e</sup>
Profenofos	3.91±1.34 <sup>G</sup>	2.99±0.58 <sup>d</sup>	35.33±0.05 <sup>h</sup>	39.50±0.43 <sup>f</sup>
Propolis	15.67±0.73 <sup>c</sup>	28.30±0.94 <sup>a</sup>	80.49±0.10 <sup>b</sup>	97.31±0.38 <sup>a</sup>
Ginseng	26.37±0.38 <sup>c</sup>	27.01±0.82 <sup>a</sup>	81.42±0.18 <sup>a</sup>	96.25±0.92 <sup>a</sup>
Chlorpyrifos +Propolis	29.20±0.68 <sup>b</sup>	26.03±1.56 <sup>a</sup>	72.55±0.14 <sup>c</sup>	92.41±0.29 <sup>b</sup>
Chlorpyrifos +Ginseng	31.20±0.37 <sup>a</sup>	22.77±1.19 <sup>b</sup>	71.48±0.09 <sup>d</sup>	97.20±0.53 <sup>a</sup>
Profenofos + Propolis	26.85±0.48 <sup>c</sup>	24.47±1.70 <sup>ab</sup>	65.13±0.05 <sup>e</sup>	89.97±0.20 <sup>c</sup>
Profenofos + Ginseng	23.40±1.01 <sup>d</sup>	23.48±2.45 <sup>ab</sup>	62.36±0.11 <sup>f</sup>	87.99±0.45 <sup>d</sup>

Means within the same column in each category carrying different litters are significant at ( $P \leq 0.05$ ) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

Table (6): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Glutathione peroxidase in male albino rats (mean ± SE). (N = 7).

Groups	Oxidative stress markers			
	plasma Glutathione Peroxidase	Liver Glutathione Peroxidase	Brain Glutathione Peroxidase	Kidney Glutathione Peroxidase
Control group	34.53±0.67 <sup>a</sup>	33.04±0.74 <sup>a</sup>	77.22±0.09 <sup>c</sup>	55.13±0.36 <sup>h</sup>
Chlorpyrifos	5.61±0.47 <sup>h</sup>	4.99±0.51 <sup>c</sup>	26.79±0.17 <sup>f</sup>	143.47±0.36 <sup>a</sup>
Profenofos	9.90±1.19 <sup>g</sup>	2.64±0.44 <sup>d</sup>	24.23±0.01 <sup>G</sup>	138.32±0.40 <sup>b</sup>
Propolis	32.14±0.45 <sup>b</sup>	34.50±1.34 <sup>a</sup>	88.46±0.38 <sup>a</sup>	66.27±0.43 <sup>g</sup>
Ginseng	30.33±0.39 <sup>c</sup>	35.89±0.82 <sup>a</sup>	80.20±0.03 <sup>b</sup>	65.25±0.92 <sup>g</sup>
Chlorpyrifos +Propolis	26.46±0.84 <sup>d</sup>	22.83±1.00 <sup>b</sup>	71.24±0.30 <sup>c</sup>	81.41±0.29 <sup>d</sup>
Chlorpyrifos +Ginseng	28.37±0.45 <sup>d</sup>	35.70±1.13 <sup>a</sup>	70.45±0.13 <sup>c</sup>	96.20±0.53 <sup>c</sup>
Profenofos + Propolis	23.86±0.49 <sup>e</sup>	29.33±1.68 <sup>ab</sup>	74.43±0.20 <sup>d</sup>	78.97±0.20 <sup>c</sup>
Profenofos + Ginseng	20.42±1.02 <sup>ef</sup>	32.34±2.46 <sup>a</sup>	71.26±0.03 <sup>c</sup>	77.00±0.44 <sup>f</sup>

Means within the same column in each category carrying different litters are significant at ( $P \leq 0.05$ ) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

Table (7): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Nitric oxide (NO) & Glucose -6-Phosphate hydrogenase (G-6-PH) in male albino rats (mean ± SE). (N = 7).

Groups	Oxidative stress markers	
	Serum NO ( $\mu\text{mol/L}$ )	Serum G-6-PH ( $\mu\text{mol/L}$ )
Control group	0.77±0.11 <sup>bc</sup>	2.18±0.01 <sup>a</sup>
Chlorpyrifos	4.52±0.05 <sup>a</sup>	0.005±0.001 <sup>c</sup>
Profenofos	5.30±0.21 <sup>a</sup>	0.006±0.001 <sup>c</sup>
Propolis	0.80±0.25 <sup>b</sup>	2.16±0.05 <sup>a</sup>
Ginseng	0.85±0.31 <sup>b</sup>	2.17±0.1 <sup>a</sup>
Chlorpyrifos +Propolis	0.83±0.22 <sup>b</sup>	2.09±0.003 <sup>ab</sup>
Chlorpyrifos +Ginseng	0.86±0.15 <sup>b</sup>	2.10±0.002 <sup>ab</sup>
Profenofos + Propolis	0.90±0.35 <sup>b</sup>	2.07±0.002 <sup>ab</sup>
Profenofos + Ginseng	0.89±0.18 <sup>b</sup>	2.08±0.005 <sup>ab</sup>

Means within the same column in each category carrying different litters are significant at ( $P \leq 0.05$ ) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

Table (8): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on plasma Acetylcholinesterase (mmol/min/ml) in male albino rats (mean ± SE). (N = 7).

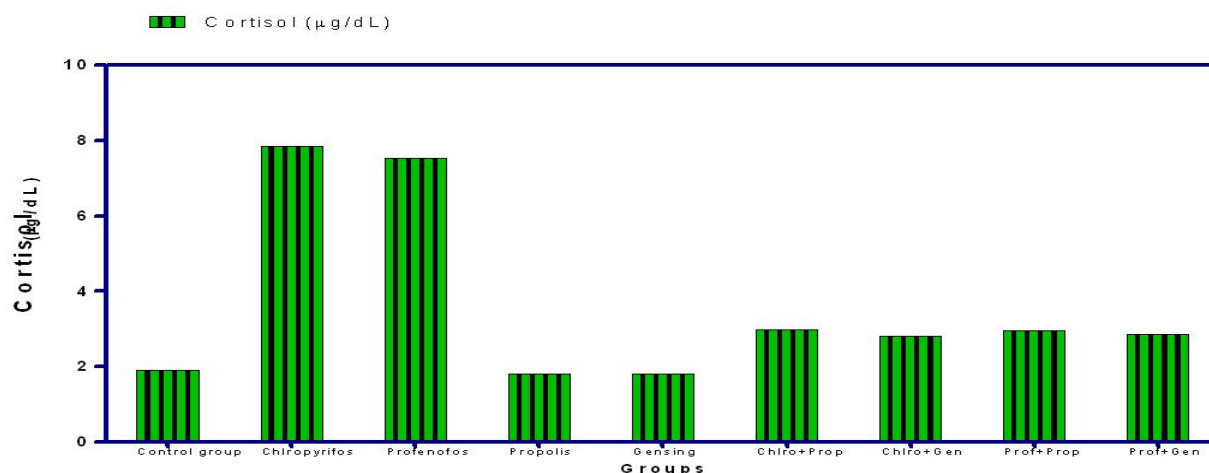
Groups	Acetylcholinesterase (mmol/min/ml)
Control group	1486.00±3.52 <sup>a</sup>
Chlorpyrifos	612.60±25.04 <sup>d</sup>
Profenofos	530.80±15.14 <sup>c</sup>
Propolis	1481.80±13.73 <sup>a</sup>
Ginseng	1476.80±7.54 <sup>a</sup>
Chlorpyrifos + Propolis	1363.20±12.13 <sup>b</sup>
Chlorpyrifos + Ginseng	1042.00±16.77 <sup>c</sup>
Profenofos + Propolis	1444.80±14.06 <sup>a</sup>
Profenofos + Ginseng	1059.80±17.48 <sup>c</sup>

Means within the same column in each category carrying different litters are significant at ( $P \leq 0.05$ ) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.

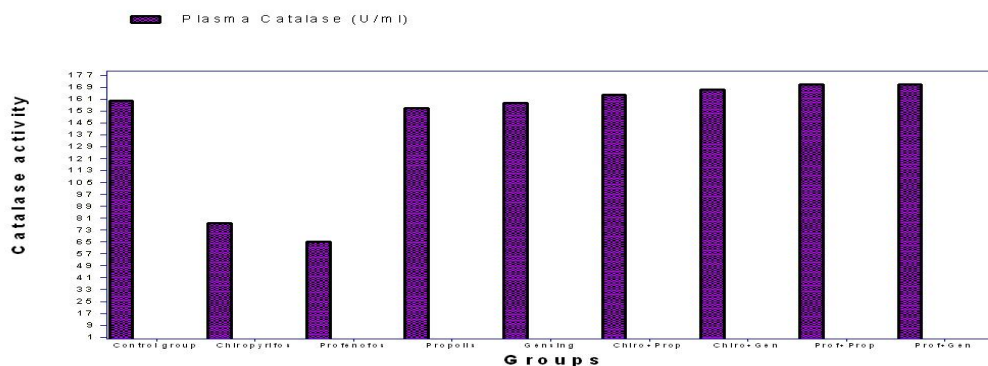
Table (9): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on plasma monamines in male albino rats (mean ± SE). (N = 7).

Groups	Dopamine (DA) (µg/ml)	Serotonin (5-HT) (µg/ml)	Epinephrine (µg/ml)	Nor-Epinephrine (µg/ml)
Control group	1.09±1.092 <sup>a</sup>	0.66±0.011 <sup>b</sup>	0.66±0.01 <sup>ab</sup>	0.95±0.021 <sup>a</sup>
Chlorpyrifos	0.35±0.060 <sup>c</sup>	0.48±0.075 <sup>c</sup>	0.42±0.096 <sup>c</sup>	0.63±0.098 <sup>c</sup>
Profenofos	0.45±0.045 <sup>c</sup>	0.47±0.098 <sup>c</sup>	0.40±0.081 <sup>c</sup>	0.60±0.097 <sup>c</sup>
Propolis	1.07±0.032 <sup>ab</sup>	0.78±0.030 <sup>a</sup>	0.65±0.096 <sup>ab</sup>	0.98±0.011 <sup>a</sup>
Ginseng	1.09±0.030 <sup>ab</sup>	0.77±0.019 <sup>a</sup>	0.66±0.082 <sup>ab</sup>	0.96±0.001 <sup>a</sup>
Chlorpyrifos + Propolis	1.10±0.099 <sup>a</sup>	0.67±0.029 <sup>b</sup>	0.69±0.097 <sup>a</sup>	0.87±0.052 <sup>ab</sup>
Chlorpyrifos + Ginseng	1.11±0.033 <sup>a</sup>	0.60±0.090 <sup>b</sup>	0.68±0.015 <sup>ab</sup>	0.83±0.043 <sup>ab</sup>
Profenofos+ Propolis	0.92±0.018 <sup>ab</sup>	0.68±0.092 <sup>ab</sup>	0.67±0.011 <sup>ab</sup>	0.85±0.032 <sup>ab</sup>
Profenofos+Ginseng	1.06±0.044 <sup>ab</sup>	0.66±0.022 <sup>b</sup>	0.70±0.079 <sup>a</sup>	0.88±0.016 <sup>ab</sup>

Means within the same column in each category carrying different letters are significant at ( $P \leq 0.05$ ) using Duncan's multiple range test, where the highest mean value has symbol (a) and decreasing in value were assigned alphabetically.



**Fig (1) :** Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on monoamines in male albino rats .



**Fig. (2):** Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Catalase activity (in plasma) in male albino rats .

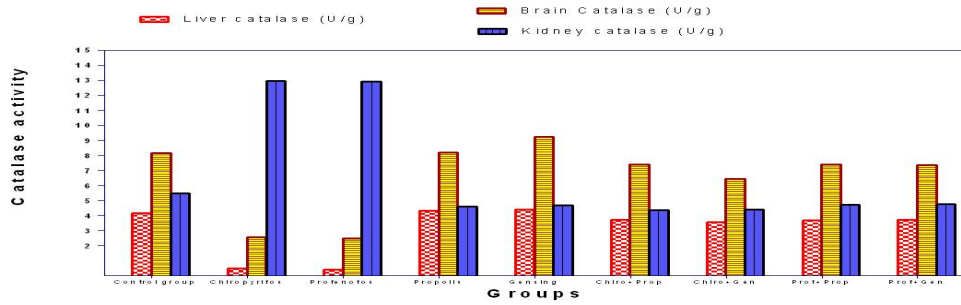


Fig (3): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Catalase activity (in tissue homogenates) in male albino rats .

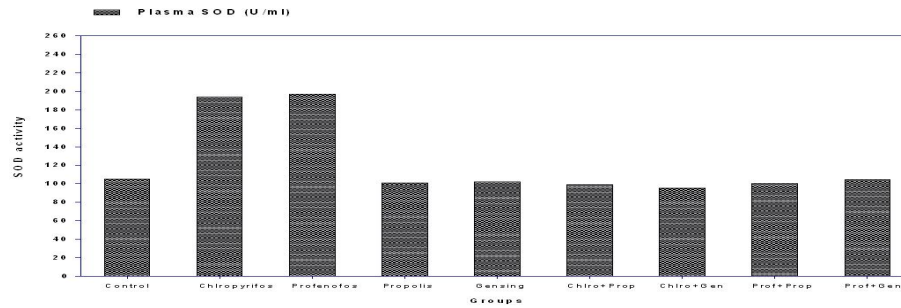


Fig (4): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on SOD (in plasma) activity in male albino rats .

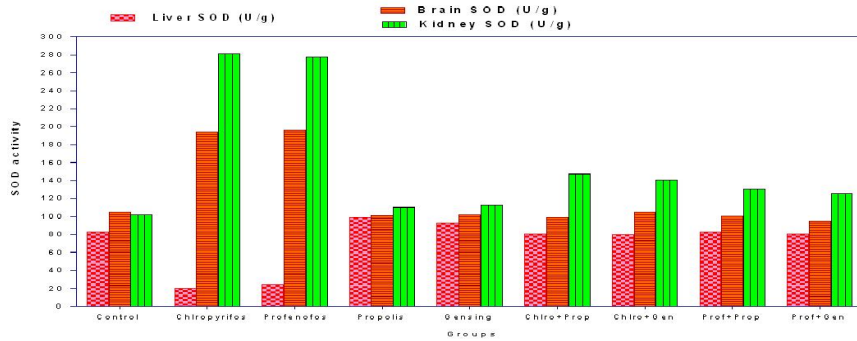


Fig (5): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on SOD activity (in tissue homogenates)in male albino rats .

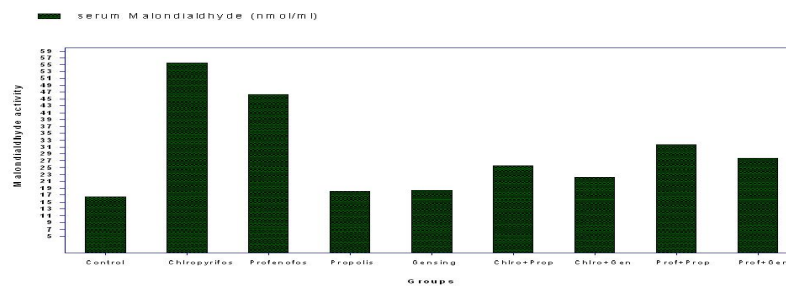


Fig (6): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Malondialdehyde (MDA) (in plasma) in male albino rats.



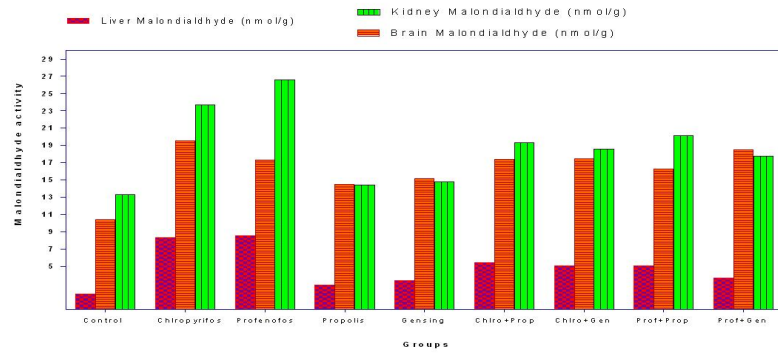


Fig (7): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Malondialdehyde (MDA) (in tissue homogenates) in male albino rats.

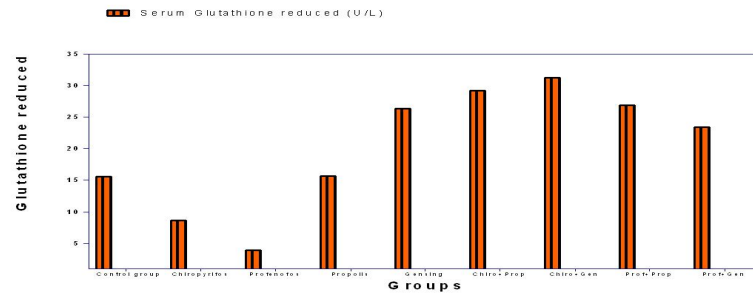


Fig (8): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Glutathione reduced (in serum) in male albino rats.

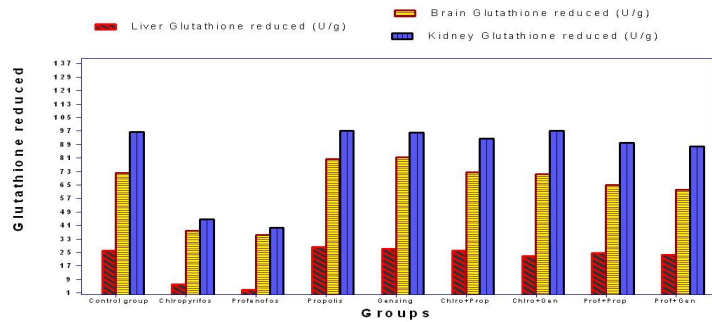


Fig (9): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Glutathione reduced (in tissue homogenates) in male albino rats.

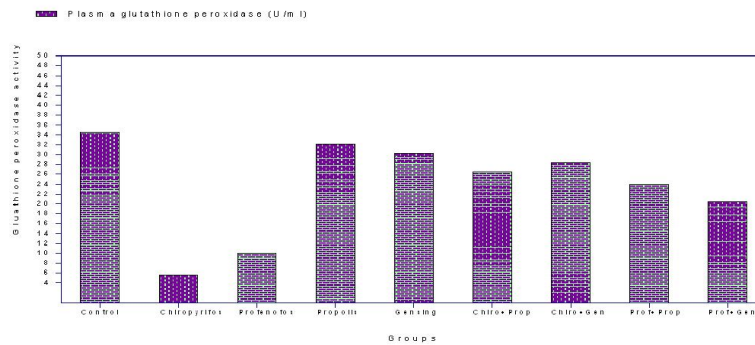


Fig (10): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Glutathione peroxidase (in plasma) in male albino rats

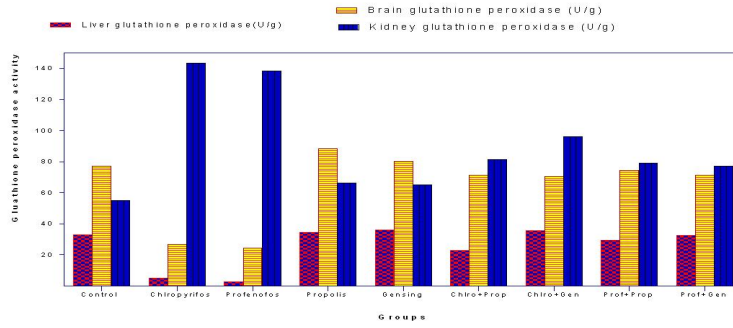


Fig (11): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Glutathione peroxidase (in tissue homogenates) in male albino rats

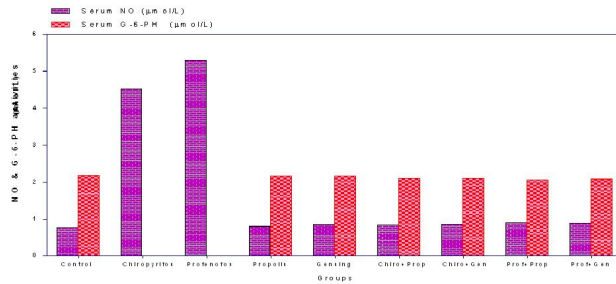


Fig (12): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on NO & G-6-PH activities in male albino rats.

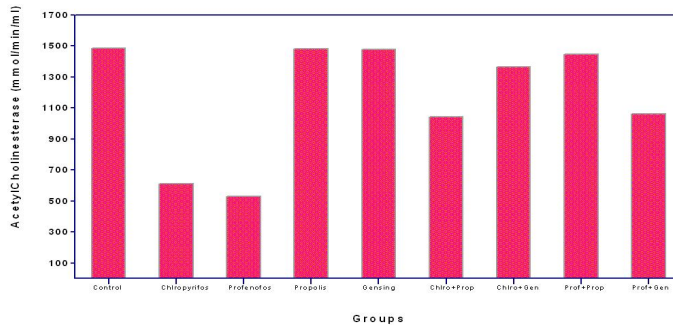


Fig (13): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on Acetylcholinesterase (mmol/min/ml) in male albino rats.

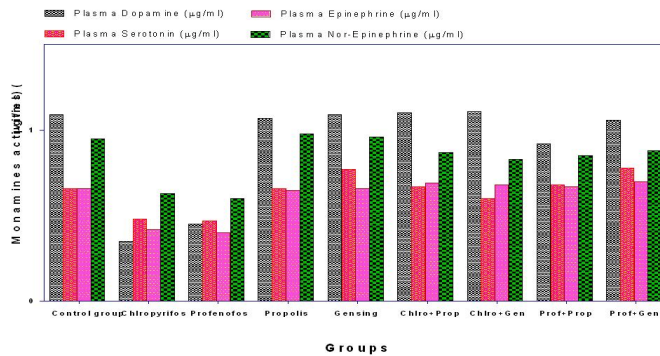


Fig (14): Effect of Chlorpyrifos (6.75 mg/kg), Profenofos (20mg/Kg) , Propolis (70 mg/ Kg), Ginseng (200 mg/Kg) and their combinations on plasma monoamines in male albino rats.

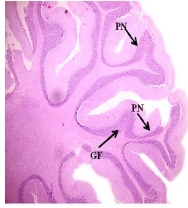


Fig. (15): Cross section of control rat brain of (Group 1) showing normal brain tissues formed of round and pyramidal shaped neurons surrounded by eosinophilic glial fibers (H and E x 400) (PN: Pyramidal neurons, GF: Glial fibers).

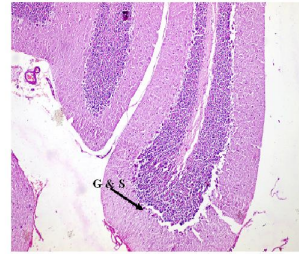


Fig. (19): Cross section of rat brain of group (4) treated with propolis (70 mg/ Kg) with normal gyri and sulci of brain tissue (H and E x 100) (G & S: gyri and sulci).

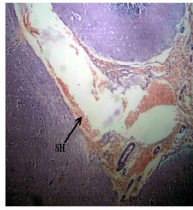


Fig. (16): Cross section of rat brain of group (2) treated with chlorpyrifos (6.75 mg/kg) showing fragments of brain tissue separated by large areas of hemorrhage (H and E x 200) (SH: Severe hemorrhage).

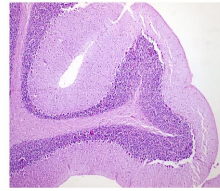


Fig. (20): Cross section of rat brain of group (5) treated with ginseng (200 mg/ Kg) showing normal brain tissue formed of round and pyramidal shaped neurons surrounded by eosinophilic glial fibers (H and E x 200) (PN: Pyramidal neurons).

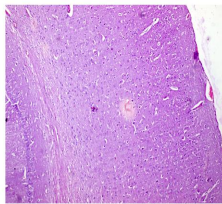


Fig. (17): Cross section of rat brain of group (3) treated with chlorpyrifos (6.75 mg/kg) showing atrophic brain tissues with few atrophic neurons with pyknotic nuclei and surrounded by excessive glial tissue (H and E x 400) (ABT: Atrophic brain tissue, PN: pyknotic nuclei).

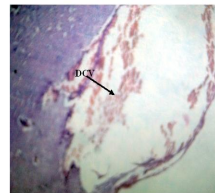


Fig. (21): Cross section of rat brain of group (6) treated with (Chlorpyrifos + Propolis) (6.75 mg/kg) & (70 mg/kg) respectively showing dilated and congested vascular space filled with few red blood cells (H&E x 200) (DCV: Dilated congested vein).



Fig. (18): Cross section of rat brain from group (3) treated with profenofos (20 mg/ Kg) showing dilated congested vascular space filled with red blood cells and compressing brain tissue (H&E x 200) (DCVR: Dilated congested vascular space filled with red blood cells).

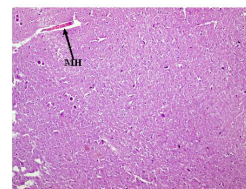
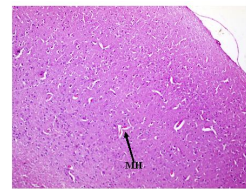


Fig. (22): Cross section of rat brain of group (7) treated with (Chlorpyrifos + ginseng) (6.75 mg/kg) & (200 mg/kg) respectively showing normal appearance of sulci of brain tissues with very mild haemorrhage in between the compartments of brain tissues (H and E x 200) (MH: Mild haemorrhage).

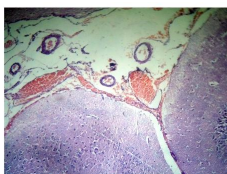


Fig. (23): Cross section of rat brain of group (8) treated with (profenofos + propolis) (20 mg/kg) & (70 mg/kg) respectively showing mild congestion with mild area of hemorrhage between the compartments of brain tissues (H&E X200) (M: Mild congestion).

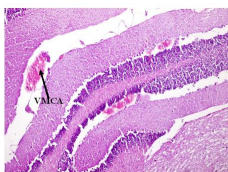


Fig. (24): Cross section of rat brain of group (9) treated with (profenofos + ginseng) (20 mg/kg) & (200 mg/kg) respectively showing normal brain tissues fragment separated by very mild congested area (H and E x 100) (V: Very mild congested area).

#### 4. Discussion:

Chlorpyrifos (CPF) is an effective organophosphate (OP) pesticide used heavily throughout the world for agriculture and domestic purposes. The main target of OP pesticides is acetylcholinesterase (AChE), which hydrolyses acetylcholine (ACh) in cholinergic synapses and at neuromuscular junctions [38]. This results in the accumulation of ACh in the synapses which in turn induces hyperactivity in cholinergic pathways.

Profenofos caused different symptoms of toxicity and revealed some biochemical changes especially in the enzymes activity of the liver and brain following two sublethal doses of profenofos in mice [39].

Natural products are a promising source for the discovery of new pharmaceuticals. In the last decades, several works dealing with propolis' composition and biological properties have been published, revealing the interest of researchers on this bee product and its potential for the development of new drugs [9].

The low cost of traditional medicinal plants also raise significant interest to prevent morbidity and mortality from chronic diseases in countries where low or middle income populations are important [40].

Increased utilization of medicinal plants became a World Health Organization (WHO) policy in 1970. Plants and herbs are chemical factories that directly provide about 25% of currently used drugs and another 25% of drugs comprise chemically altered natural products [41].

Propolis is a resinous hive product collected by honeybees from plants, showing a very complex chemical composition [10]. It has been used in folk medicine since ancient times, due to its many

biological properties, such as antibacterial [11], antitumor [42,43], and immunomodulatory [44], among others.

Ginseng is a well-known medicinal herb in traditional Asian medicine and is considered an adaptogen. *Panax ginseng* C.A. Meyer (Araliaceae), which grows in China and Korea, has a variety of beneficial biological actions that include anti-carcinogenic, anti-diabetic-inflammatory effects, as well as cardiovascular protection and neuroprotection [45, 46].

#### Effect on Cortisol level:

It was clearly evident that the administration of Chlorpyrifos and/or Profenofos each alone in their recommended doses daily for successive 60 days afforded a highly significant increase in serum cortisol level after the end of the experiment when compared with control group. Concerning the effect of either Propolis or Ginseng, our results revealed that Propolis and/or Ginseng treated groups showed non significant changes in serum cortisol level when compared with control group. A non significant increase in serum cortisol level was also recorded in response to treatment of male rats with the combinations of either Chlorpyrifos or Profenofos with either Propolis or ginseng compared with normal control group.

Cortisol, a corticosteroid hormone, is considered to be an important physiological effector of homeostasis in all vertebrates, through its effects on metabolism and immune function. [47] stated that the decrease of normal cortisol secretion is due to the effects of Organophosphorous on cortisol biosynthesis pathway [48] found that an increase in gonadal oxidative stress is generally coincident with decreased levels of  $\beta$ -estradiol.

One of the characteristics of organophosphorous pesticides is induction of stress. Stress is a response to every situation which threatening homeostasis and result in activation of hypothalamic-pituitary-adrenal (HPA) axis and sympathetic autonomic nervous system which consequently lead to hyperglycemia [49].

Activation of HPA (Hypothalamic-pituitary-adrenal) axis causes secretion of glucocorticoids from adrenal cortex. Cortisol increases blood glucose by induction of gluconeogenesis pathway as a result of organophosphorous exposure [50].

#### Effect on antioxidant activity:

Regarding the effect of profenofos and chlorpyrifos on catalase activity of normal rats, chlorpyrifos and profenofos afforded a marked decrease in plasma, liver and brain catalase after the end of the study when compared with control group, whereas, a significant increase in the enzyme activity was recorded in kidney. Treatment of normal rats with either propolis or Ginseng alone exhibited non significant changes in Catalase of liver and plasma after the end of the experiment when compared with control group ,

Whereas, a significant increase was reported in brain and kidney tissues respectively compared with control group. While combinations of Chlorpyrifos, Profenofos with either Propolis or ginseng exhibited a significant decrease in Catalase activity of liver, kidney, plasma and brain after the end of the study except combinations of Chlorpyrifos with either propolis and/or ginseng in the plasma which showed a non significant increase compared with normal control group. The results of our study revealed that treatment of normal rats with either Chlorpyrifos or profenofos elicited a highly significant decrease in serum and liver SOD level after the end of the study together with a marked increase in SOD activity of kidney and brain when compared with control group. Treatment of normal rats with either propolis or ginseng for 8 weeks elicited a significant increase in SOD activity of the liver, Kidney and serum after the end of the study except with ginseng in serum which showed a significant increase compared with control group. Whereas, the combinations of the plant extracts with the test insecticides afforded a significant decrease in SOD activity of the brain and serum, beside a significant increase and non significant changes in the SOD activity of the kidney and liver respectively compared with normal control group. The MDA content of the serum, liver, kidney and brain were significantly elevated in response to treatment of normal male rats with either Chlorpyrifos or profenofos for 8 weeks compared with normal control group. The same previous response was reported with propolis, ginseng and their combinations with either Chlorpyrifos or profenofos compared with control group.

It was apparent from our results that treatment of rats with Chlorpyrifos, Profenofos each alone afforded a significant decrease in serum, liver, kidney and brain reduced glutathione after the end of the study when compared with normal control group. On the other hand, the results revealed that Ginseng and/or Propolis induced a non significant change in reduced Glutathione content of the kidney, liver and serum together with a significant increase in the reduced glutathione content of the brain compared with control group.

The plasma Glutathione peroxidase level was significantly reduced in all groups treated with Chlorpyrifos, Profenofos each alone, propolis, ginseng and their combinations for successive 60 days when compared with normal control group. Whereas, a significant increase was recorded in the enzyme activity of the kidney of all treated groups when compared with control group. The enzyme activity was significantly decreased and increased in the brain tissue in response to treatment with the insecticides and plant extracts used compared with the control group. Together with a significant decrease in the enzyme activity in response to combinations of propolis and/or

ginseng with either Chlorpyrifos or profenofos. The enzyme activity in the plasma was markedly decreased in response to treatment with either Chlorpyrifos or profenofos compared with control group. Beside a non significant increase in response to treatments with either propolis or ginseng. Whereas, a non significant change was reported in response to treatments with all combinations used except combination of Chlorpyrifos with propolis which showed a significant decrease compared with normal control group. The serum Nitric oxide and glucose – phosphate dehydrogenase level were significantly elevated in serum, liver, brain and kidney in groups treated with Chlorpyrifos and /or Profenofos each alone. Whereas, Treatment of normal rats with Ginseng, Propolis and their combinations with both Chlorpyrifos and Profenofos afforded non significant changes after the end of the study when compared with normal control group.

Pesticides may also affect the biochemical and physiological functions in living organisms, thereby affecting the membrane integrity [51] and may induce in vivo and in vitro generation of reactive oxygen species (ROS) leading to oxidative stress.

Oxidative injury, resulting from excessive release of free radicals, likely contributes to the initiation and progression of brain injury. Therefore, antioxidant therapies as propolis treatment aimed at reducing oxidative stress have received considerable attention [52].

Lipid peroxidation (LPO) is one of the molecular mechanisms involved in pesticide toxicity. Living organisms have a complex antioxidant (enzymatic and non-enzymatic) system to protect against the deleterious effects of free radicals. Activity of the antioxidant defense system can be increased or inhibited under chemical stress, and antioxidant parameters therefore represent biomarkers of interest [53]. The enzymes that provide the first line of defense include superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR). Reduced glutathione (GSH) is the primary cellular antioxidant (non-enzymatic) and plays an important role in the antioxidation of ROS and free radicals and, as a thiol- containing co-enzyme, in the detoxification of xenobiotic compounds. Glutathione-S transferase (GST) is a group of multifunctional enzymes that catalyze the conjugation of GSH with a variety of electrophilic metabolites that are involved in the detoxification of both reactive intermediates and oxygen radicals [54].

Our results are reinforced by *Verma & Srivastava (55)*. They reported that Chlorpyrifos is known to produce oxidative stress resulting in the accumulation of lipid peroxidation products in different organs of rats, also authors *Shadina et al., (56)* reported that CPF and other OP pesticides have been shown to damage DNA also. The generation of free

radicals constitutes one of the underlying mechanisms of Chlorpyrifos and Profenofos intoxication. Changes in blood MDA levels and SOD, CAT, and GSH-Px activities have been determined to develop due to the generation of free radicals. Since the generation of free radicals also causes red blood cell damage, damage occurs in tissues, primarily in the liver. Pesticides are reported to inhibit the enzymatic defense also in rat tissues [57]. Thus it can be concluded that these OP pesticides generate oxidative stress by inhibiting both enzymatic and non enzymatic antioxidant defenses. The generated oxidative stress may contribute substantially in the overall toxicity of OP pesticides.

CPF exposure caused an increase in levels of lipid peroxidation in the liver, kidney, spleen, and brain of rats as measured by estimation of thiobarbituric acid reactive substances (TEARS). The increase in the level of TEARS ranged from 30% to 74% in different tissues of rats treated with 100mg CPF/kg body weight for 3 days. When rats were exposed to CPF following the treatment with vitamins, the TEARS level remained almost unaltered in the liver and kidney but was slightly elevated in the brain and spleen of the same animals [55].

The results of *Ehrhart and Zeevalk, (58)* suggest that ginseng root extract could protect astrocytes from oxidant stress generated by  $H_2O_2$  which is consistent with the reports of antioxidant effects observed in ginseng root extracts in other cellular types. Panax ginseng saponins have shown a suppressive action on the lipid peroxidation caused by radical generating systems in tissue preparations, and attenuate lipid peroxidation in the rat liver homogenate. In addition, *Yousef and Salama, (59)* found that propolis water extract can protect brain cells against oxidative stress-induced death and may exert neuroprotective effects through their antioxidant actions.

*Naval et al., (60)* have demonstrated the protective effect of a normalized aqueous Panax ginseng root extract on hydrogen peroxide-induced oxidative damage in astrocytic primary cultures. Their results showed that the root of Korean ginseng is endowed with significant antioxidant properties and this is the base for its glioprotection against acute oxidant stress.

#### **SOD and CAT:**

Our results were in complete accordance with previous findings, but our results showed significant increase in Catalase enzyme in kidney homogenates and as it is well known fact that CAT is the enzyme involved in the breakdown of  $H_2O_2$  so this explain the reason of increasing CAT in kidney as the elevation of CAT may be due to a counteraction to scavenge the damaging toxic free radicals which may cause tissues injury and damage. CAT is mainly located in the peroxisomes and is responsible for the reduction of

hydrogen peroxide produced from the metabolism of long chain fatty acids in peroxisomes to water and oxygen [61].

In contrary to our result, CAT and SOD activities in liver were increased in chlorpyrifos treated mice probably to dismutate superoxide anions and to decompose  $H_2O_2$  according to (*Nagat Aly, et al., [62]*). These data are parallel with *Yu et al.[63]*. In contrast. *Bindhumol et al., (64), Banudevi et al.(2006)* found that bisphenol A and PCB's decreased the activity of both CAT and SOD. Also, our results revealed that CAT enzyme was decreased in brain tissues and this disagrees with *Verma et al.,(65)* as they reported that the activities of SOD and CAT were significantly inhibited in all the tissues tested (except SOD in brain) to varying extents, however, pre-treatment with vitamins prevented CPF induced changes. In addition, *Sayed et al., (66)* reported that organophosphorous exposure also causes significant decreases in CAT activities in liver, kidney and gill tissues of *Channa punctatus*.

Our results coincide with *Kono and Fridovich, (67)*. They reported that the inhibition of CAT activity could be due to the flux of superoxide radicals, resulting in  $H_2O_2$  increase in the cell

SOD help to dismutase superoxide radical  $O_2^-$  to hydrogen peroxide ( $H_2O_2$ ) [68], Thus the increase in CAT activities in the kidney may be in response to  $H_2O_2$  produced by SOD activity since CAT is responsible for the detoxification of  $H_2O_2$  to water.

Our results were greatly reinforced by *Mates, (69)* who reported that SOD has been strongly inhibited in brain, this may be due to over production of free radicals. Moreover, the brain is especially vulnerable to oxidative damage because of its high abundance of polyunsaturated fatty acids, and has a relatively low antioxidant defense system. The cause of tissue differences could be due to different rates of free radical generation and different antioxidant potentials in the tissues. At the same time, the results of *Lin et al., [70]* were in full agreement with our results (increasing the levels of SOD in brain and kidney) as they reported that profenofos increased the antioxidant activities (SOD, CAT,) earlier than the decrease of ChE activity. They suggested that profenofos can result in the increase of the antioxidant enzyme activities which may be earlier diagnostic index in profenofos poisoning.

In accordance with our results, Propolis also induces the activation of antioxidant enzymes such as Superoxide dismutase and catalase (CAT) against free radicals. The antioxidant activity seemed to relate with total flavonoid contents of the extract. Flavonoids are reported to be the most abundant and most effective antioxidant in propolis [71].

Our results were in full agreement with *Naval et al., (60)*. They reported that Ginseng extract had a significant effect on the reduction of astrocytic death

induced by H<sub>2</sub>O<sub>2</sub>. Dose–response experiments revealed that this ginseng extract increased cell viability at a wide range of concentrations. Therefore, they investigated the effects of this extract on antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione peroxidases (GPx) and glutathione reductase (GR), on glutathione content (reduced and oxidized forms and red/ox index) and on the intracellular reactive oxygen species (ROS) formation. Exposure of astrocytes to H<sub>2</sub>O<sub>2</sub> decreased the activities of antioxidant enzymes, and increased ROS formation. Ginseng root extract reversed the effect of almost all of these parameters in H<sub>2</sub>O<sub>2</sub>-injured primary cultures of rat astrocytes.

It was shown that administration of ginseng in humans for 8 weeks decreased MDA while increased SOD and CAT activities. *Hyunghie et al.*, (72) suggested that ginseng administration elevated antioxidant potential by decreasing MDA level and by increasing SOD and CAT activities as scavengers.

#### MDA:

Levels of MDA, a major oxidation product of poly-unsaturated fatty acids, have been considered to be the most significant indicator of membrane lipid peroxidation arising from the interaction of reactive oxygen types with cellular membranes [73].

*Kumar and Ramakrishna* (5) have reported an increase in MDA levels in chronic Chlorpyrifos intoxication and these findings were in complete accordance with our results.

Increasing level of MDA in our study as a result of treatments with both Chlorpyrifos and profenofos go hand in hand with the results of *Mecdad et al* (74). They revealed statistically significant reduction of antioxidant defense enzymes, total antioxidant capacity, while MDA levels showed significant elevations in MDA in insecticides exposed workers.

Furthermore, Exposure of rats to Chlorpyrifos through drinking water resulted in a significant increase in lipid peroxidation and protein oxidation as indicated by the significant increase in MDA content, protein carbonyls and apoptosis levels suggesting that Chlorpyrifos activated the formation of free radicals in cerebral cortex tissue. This is corroborated with the findings which demonstrated that Chlorpyrifos exposure stimulated the generation of reactive oxygen species (ROS) in the brain [75].

Our results weren't in agreement with some authors who have underlined the occurrence of alterations in enzyme activities and MDA levels upon the administration of propolis. *Jasprica et al.*,(76) have reported Propolis to cause reduction in MDA levels.

#### G6PD:

In contrary to our results, Sub-lethal exposure to Chlorpyrifos caused a significant inhibition in G6PD activity. It is an important enzyme of hexose monophosphate shunt and its function in the mature

cells is to generate NADPH, while it is required for conversion of oxidized glutathione to reduced glutathione form that in turn is necessary for membrane integrity of cell membranes. This might be the possible reason for the increased fragility of cells upon treatment with different pesticides. It is well documented in literature that treatment with Organophosphorous insecticides such as Chlorpyrifos results in a decrease in GSH levels, thereby decreasing G6PD activity [65].

#### Glutathione:

GSH is an important naturally occurring antioxidant, which prevents free radical damage and helps detoxification by conjugating with chemicals. In addition, GSH is central to the cellular antioxidant defenses and acts as an essential cofactor for antioxidant enzymes including GPx, GR and GST. Under oxidative stress, GSH is consumed by GSH related enzymes to detoxify the peroxides produced due to increased lipid peroxidation [77].

Our results showed marked decrease in glutathione enzyme and these results were in full agreement with *Rana et al.*, (78). They reported that glutathione deficiency contributes oxidative stress, which plays a key role in aging and the pathogenesis of many diseases including seizures, Alzheimer's disease, Parkinson's disease, liver disease, cystic fibrosis, sickle cell anemia, AIDS, cancer, heart attack, stroke and diabetes. GSH is also a substrate of enzymes, glutathione peroxidase and glutathione-S-transferase.

It has been reported also that the long-term treatment with OP causes a gradual depletion of GPx, and GST [79].

Our results were compatible also with *Fang et al.*, (80). They reported that a considerable decline in GSH content in the tissue may be due to its utilization to challenge the prevailing oxidative stress under the influence of ROS generated from MP and CPF oxidative stress. However, oxidative stress can induce GSH rising by protective role in the organisms exposed to chemicals. Reduced GSH and its metabolizing enzymes provide the foremost defense against ROS-induced cellular damage.

Meanwhile, the administration of profenofos caused a significant decrease in the levels of glutathione peroxidase (GPx) and reduced glutathione (GSH), and an increase in the lipid peroxidation (LPO) level [81].

Similar results were recorded by [82]. They reported that inhibition of GST was observed in brain

Furthermore, a reduction in GSH levels led to a significant induction of GST activity during most of the exposure period. GST has been strongly inhibited by exposure to profenofos, whereas GST was induced in fish after exposure to profenofos [83].

Similar kind of results (inhibition in GR) and GSH depletion were observed in fish after exposure to profenofos. Glutathione was depleted in all the tissues,

an indication of severe oxidative stress, and may lead to lipid peroxidation and tissue damage. Glutathione is the major low-molecular-weight, non-enzymatic antioxidant [23].

#### **Nitric oxide (NO):**

Concerning the effect on NO production, our results are greatly reinforced by *Orsi et al., (84)*. They demonstrated that caffeic acid (The main component of propolis) can act as a prooxidant and an effective irreversible inhibitor of glutathione S-transferases that causes a decrease of generation of NO by activated macrophage.

#### **Effect on Acetylcholinesterase enzyme activity:**

It was clear from our results that the administration of either Chlorpyrifos or Profenofos each alone to normal rats for successive 60 days afforded a marked decrease in serum acetylcholinesterase activity after two months post administration. Whereas, a non significant change in serum Acetylcholinesterase activity was observed in the groups treated with either Propolis or Ginseng.

At the meantime, a significant decrease was recorded in the groups treated with the combinations of the insecticides used with either Propolis or ginseng after 8 weeks post administration except the combination of Profenofos with Propolis which showed a non significant change compared with control group. Yet the results of the drugs combinations were much higher than that produced with the insecticide alone indicating a good ameliorating effect with the used plant extracts.

OP pesticides compounds are known to induce toxicity in mammals by inhibiting acetylcholinesterase (AChE), which leads to the accumulation of acetylcholine and the subsequent activation of cholinergic muscarinic and nicotinic receptors. OP pesticides are also known to inhibit pseudocholinesterase activity. Other systems that may be affected by OP pesticide exposure are the immune system [85], pancreas [86], liver, Kidney [87], hematological system [88] and reproductive system [89].

The main target to OP pesticides is Acetylcholinesterase (ACHE) which hydrolyses acetylcholine (ACh) in cholinergic synapse and in neuromuscular junctions where this enzyme plays a key role in cell to cell communication, [90].

Our results were in full agreement with *Abbassy et al., (91)*. They showed that plasma cholinesterase activity of rats fed single doses of Chlorpyrifos (32 mg/kg body) was significantly reduced after 24 hours post feeding. CPF also inhibits ACHE irreversibly. It can cause acute poisoning and well known symptoms include myosis, increased urination, diarrhea, diaphoresis, lacrimation and salivation.

On the same basis, CPF is metabolized by microsomal mixed function oxidase system to active

oxon metabolites which are more potent inhibitor of acetylcholinesterase. Besides being potent anticholinesterase compound, CPF is reported to affect adversely the membrane signal transduction, brain development, neurotransmitter receptor and release of neurotransmitters. CPF is also a potent anticholinergic agent and affects other metabolic events, like changes in membrane lipid physicochemical properties, monoaminergic system, and reproductive system and DNA damage [92].

The toxicity of Chlorpyrifos is attributed to inhibition of the enzyme acetylcholinesterase and it is well known that the classical role of acetylcholinesterase is to hydrolyze the neurotransmitter acetylcholine, effectively clearing it from the synapse and terminating impulse conduction. There is, however, a growing body of literature suggesting a role for acetylcholinesterase and butyrylcholinesterase in development [93].

#### **Effect on plasma monoamines activity:**

Treatment of normal rats with either Chlorpyrifos or Profenofos for 60 successive days in their recommended doses elicited a marked decrease in plasma dopamine level after 8 weeks post administration when compared with normal control group. Propolis and Ginseng treated groups showed non significant changes in plasma dopamine activity after two months of administration when compared with normal control group. Yet their combinations with either Chlorpyrifos or profenofos elicited a significant increase in plasma dopamine concentration when they were compared with each insecticide alone reverting their values to nearly control values.

The administration of Chlorpyrifos and/or Profenofos in their recommended doses for successive 60 days into normal rats elicited a significant decrease in plasma serotonin level compared to normal control group. Whereas a significant increase was observed in the groups treated with either Propolis or Ginseng alone. Whereas, the combinations of either propolis or ginseng with either Chlorpyrifos or profenofos afforded non significant changes when compared with normal control group, indicating that they reverted the serotonin level to nearly its normal control level. Concerning the effect of Chlorpyrifos and Profenofos on serum epinephrine, Chlorpyrifos and/or Profenofos each alone afforded a significant decrease in serum epinephrine level when compared with normal control group after 8 weeks of the insecticides administration.

Meanwhile, treatment with either Propolis or Ginseng and their combinations with either Chlorpyrifos or profenofos revealed non significant changes after 8<sup>th</sup> weeks when compared with normal control group. The administration of either Chlorpyrifos and/or Profenofos each alone in their recommended doses for successive 60 days into normal rats elicited a



significant decrease in plasma norepinephrine content when compared with normal control group.

Meanwhile, non significant changes were reported in groups treated with either Propolis or Ginseng each alone and their combinations with either Chlorpyrifos and/or Profenofos after the end of the experiment when compared with normal control group.

However, these values were significantly elevated when compared with the groups given the insecticides used alone, indicating a potent ameliorative effect of the test plant extracts. On the same basis, our results were supported by *Parvez & Raisuddin (94)* Changes in plasma neurotransmitter levels of the treated groups might be due to the selective block plasma membrane reuptake of these amines. It is known that, plasma membrane uptake process is the key for termination of many neurotransmitters as well as, the allowance for their reuse. As recorded by several investigators, catecholamine are 12 TMD (transmembrane-spanning domain) protein that driven by inward of gradient  $\text{Na}^+$  and the transporters for NE and DA require  $\text{Na}^+$  which may be altered by Organophosphorous including Chlorpyrifos. Concerning the MAO activity, the present study revealed that chlorpyrifos treatment caused a decrease in MAO activity in most of animals. Furthermore, several lines of evidence indicate that organophosphorous compounds are strong inducers for catecholamine release.

In contrary to our results, the significant increase in brain serotonin observed following Chlorpyrifos administration might be attributed to the fact that liver damage induced by organophosphorous insecticides evidenced in our study increased ammonia concentrations which could lead to increased brain uptake of the aromatic amino acid as tryptophan. Since tryptophan is the amino acid precursor of serotonin, its hydroxylation is the rate-limiting step in serotonin synthesis and in turns increased brain serotonin synthesis [95].

Our results also were consistent with *Aldridge et al., (96)*. They showed that Chlorpyrifos exposure during early and late gestation have also shown to elicit both short- and long-term changes in serotonin (5HT) systems, disrupting the ability of 5HT to modulate adenylyl cyclase. The elevated levels in plasma catecholamines (DA & NE) could be attributed to the effect of Chlorpyrifos on chromaffin cells which secrete catecholamines (DA & NE) that might be increased after Chlorpyrifos treatment and this disagree with our obtained results. Moreover, Chlorpyrifos can elicit vigorous autonomic and neuroendocrine response due to an indirect action on the hypothalamic-pituitary adrenal axis leading to increased plasma corticosteroid levels and, catecholamines content. In contrast to our obtained results, *Santoni et al., (97)* reported that

organophosphorous compounds induced marked and long lasting increase in epinephrine and nor epinephrine plasma concentrations.

At the same time, Ginsenoside Rgi was shown to interrupt dopamine-induced elevation of reactive oxygen species (ROS) or NO generation [98].

Our results were supported by *Kim et al., (99)*. They reported that ginsenosides Rb1, Rg1, Rc, and Re inhibited tyrosine hydroxylase activity and exhibited anti-dopaminergic action since they reduced the availability of dopamine at presynaptic dopamine receptors.

## 5. Conclusions

From the obtained results, we report that both organophosphorous insecticides either Chlorpyrifos or profenofos have very dangerous and toxic effects, since they showed many side effects represented by high level of antioxidant enzymes and decreasing acetylcholinesterase enzyme. Moreover, the damage in tissues of brain.

## 6. Recommendations

So we recommend the use of the combination of propolis and ginseng which is known as antioxidants compounds in order to ameliorate the possible side effects caused by insecticides that we exposed to them to avoid the proven hazardous effect of insecticides on biochemical parameters and to overcome the side effects of both Chlorpyrifos and profenofos on liver.

## Conflict of interest

The authors declare that there are no conflicts of interest.

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## References

- [1] **Casida, J.E., and Quistad, G.B., (2004):** Organophosphate toxicology: safety aspects of nonacetylcholinesterase secondary targets, *Chemical Research in Toxicology*; 17:983–998.
- [2] **Mccopy, M.A., Reily, G.A.C., and O'Bycle, J.D., (1989):** Carbofuran poisoning in cats. *Vet. Record*. 134.; 10:255-256.
- [3] **Ecobichon, D.J., (1996):** Toxic effects of pesticides, in: C.D. Klaassen (Ed.), *Casarett and Doull's Toxicology. The Basic Sciences of Poisons*, fifth ed., McGraw-Hill, New York, pp. 643–689.
- [4] **Gomes, J., Dawodu, A.H., Lloyd, O., Revitt, D.M., and Anilal, S.V., (1999):** Hepatic injury and disturbed amino acid metabolism in mice following prolonged exposure to organophosphorus insecticides. *Hum Exp Toxicol*; 18(1):33-7.
- [5] **Kumar, A., and Chapman, J.C., (2002):** Profenofos toxicity to the eastern rainbow fish (*Melanotaenia duboulayi*). *Environ Toxicol Chem.*; 17:1799–1806.
- [6] **Prabhavathy, E.L., Meistrich, M.L., and Bairy, K.L., (2006):** Cytotoxicity and genotoxicity induced by pesticides

- profenofos on cultured human peripheral blood lymphocytes. *Drug.Chem.Toxicol.* 3:313-322.
- [7] **Saeed, R. M., Al-Koly, M. A., and Ali, M. A., (1995):** Hepatic, renal and pulmonary responses in pregnant mice and their fetuses induced by profenofos. *Journal of Union of Arab Biologists: Zoology.*; 3(A):359-386.
- [8] **Mani,F.,Damasceno,H.C.R.,Novelli,E.L.B.,Martins,E.A.M.,and Sforcin,J.M., (2006):** Propolis: Effect of different concentrations, extracts and intake period on seric biochemical variables, *Journal of Ethnopharmacology.*; 105:95-98.
- [9] **Sforcin José Maurício., and Vassya Bankova., (2011):** Review, Propolis: Is there a potential for the development of new drugs? *Journal of Ethnopharmacology.*; 133: 253–260.
- [10] **Bankova, V.S., Castro, S.L., and Marcucci, M.C., (2000):** Propolis: recent advances in chemistry and plant origin. *Apidologie.*; 31:3-15.
- [11] **Sforcin, J.M., Fernandes Jr., Lopes, C.A.M., Bankova, V., and Funari, S.R.C., (2000):** Seasonal effect on Brazilian propolis antibacterial activity. *Journal of Ethnopharmacology.*; 73: 243–249.
- [12] **Bazo, A.P., Rodrigues, M.A.M., Sforcin, J.M., Camargo, J.L.V., Ribeiro, L.R., and Salvadori, D.M.F.,(2002):** Protective action of propolis on the rat colon carcinogenesis. *Teratogenesis, Carcinogenesis and Mutagenesis.*; 22:183–194.
- [13] **Murad, J.M., Calvi, S.A., Scares, A.M.V.C., Bankova, Y., and Sforcin, J.M., (2002):** Effects of propolis from Brazil and Bulgaria on fungicidal activity of macrophages against *Paracoccidioides brasiliensis*. *Journal of Ethnopharmacology.*; 79:331-334.
- [14] **Khalil, M.L., (2006):** Biological activity of bee propolis in health and disease. *Asian Pac.J. Cancer Prev.*; 7: 22-31.
- [15] **Sung,Heungsup.,Jung.,You-Sun and Cho., Young-Keol., (2009):**Beneficial Effects of a Combination of Korean Red Ginseng and Highly Active Antiretroviral Therapy in Human Immunodeficiency Virus Type 1- infected Patients. *Clin. Vaccine Immunol.* 16 (8): 1127–31.
- [16] **Mansour, S.A., and Mossa, A.M., Lipid peroxidation and oxidative stress in rat erythrocytes induced by chlorpyrifos and the protective effect of zinc. *Pesticide Biochem. Physiol.*; 93 (2009) 34-39.**
- [17] **Mahmut Selvi., Rabia Sarıkaya., Figen Erkoç., Oner Koçak.,** Investigation of acute toxicity of chlorpyrifos-methyl on guppy *Poecilia reticulata*. *Chemosphere.*; 60(2005)93–96.
- [18] **Whitney, K.D., Seidler, F.J., Slotkin, T.A.,** Development neurotoxicity of chlorpyrifos: cellular mechanisms toxicol. *Appl. Pharmacol.*; 134 (1995)(53-62).
- [19] **Weil, C.S.,** Tables for convenient calculation of medium effective dose (LD50) or ED50 and instruction in their use *Biometrics*, 8(1952)263 -294.
- [20] **Andreson, R.A., Aaraas, I., Gaare, G., Fonnun, F.,** Inhibition of acetylcholinesterase from different species by organophosphorus compounds, carbamates and methylsulphonylflouride. *Genetic Pharmac.*; 8 (1977)331-334.
- [21] **Newairy, A.S., Salama, A.F., Hussien, H.M., Yousef, M.I.,** Propolis alleviates aluminium-induced lipid peroxidation and biochemical parameters in male rats. *Food Chem Toxicol.*; 47(2009)1093-8.
- [22] **Yousef, M.I., Salama, A.J.,** Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. *Food Chem. Toxic.*; 47(2009) 1168-1175.
- [23] **Zhang, Z., Lian, X.Y., Stringer, J.L.,** Protective effects of ginseng components in a rodent model of neurodegeneration. *Annals of Neurology.*; 57(2005) 642-648.
- [24] **Scherners, S.,** The blood morphology of laboratory animals . Blackwell Scientific Publication (3<sup>rd</sup> Ed); (1967)20:22.
- [25] **Arakawa, H., Maeda, M., and Tsujia, N., (1979):** Chemiluminescence enzyme immunoassay of cortisol using peroxidase as label *Anal. Biochem.*; (97): 248-254.
- [26] **Habig, W., and Pabast, M., Jakoby, W.j.,** The first step in mercapturic acid formation. *Biol.Chem.*; 249(1974) 7130-7139.
- [27] **Aebi, H., (1984):** Catalase in vitro. *Method Enzymol.*; 105: 121–126.
- [28] **Nishikimi, M., Roa, N.A., and Yogi, K (1972):** The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Bioph. Res. Common.*; 46(2): 849 —854.
- [29] **Beutler, F., Duron, O., and Kelly, M.B., (1963):** Improved method of estimation of blood glutathione. *J. Lab Clin. Med.*; 61(5): 882.
- [30] **Paglia, D.E., and Valentine, W. N.,** Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.*; 70(1967) 158—169.
- [31] **Ikawa, H., Ohishi, W., Yagi, K., (1979):** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.*; 95(2) 351-358.
- [32] **Montgomery, H. A. C., and Dymock, J. F., (1961):** The determination of nitrite in water. *Analyst* 86: 414–416.
- [33] **Kornberg, A., (1955):** *Methods in Enzymology.* Academic press, New York; P.323.
- [34] **Ciarlone, A.F.(1978):**Further modification of a fluorometric method for analyzing brain amines. *Microchemical J.*; 23: 9-12.
- [35] **Ellman, G.L., Courtney, K.D., Andres, Jr.,and Featherstone, R.M., (1961):** A new and rapid colorimetric of acetylcholinesterase activity. *Biochem Pharmacol.*; 7:88–95.
- [36] **Carleton, H.M.,** *Carleton’s Histological Technique .* (4<sup>th</sup> Ed), Pub .London, New York, Toronto, Oxford university press (1967).
- [37] **Snedecor, G.W., and Cochran, W.G.,** *Statistical Methods* (8<sup>th</sup>Ed), Ames Iowa State University (1982).
- [38] **Ecobichon, D.J.,** Toxic effects of pesticides, in: C.D. Klaassen (Ed.), *Casarett and Doull’s Toxicology. The Basic Sciences of Poisons*, fifth ed., McGraw-Hill, New York, pp. (1996) 643–689.
- [39] **Saeed, R. M., Al-Koly, M. A., and Ali, M. A.,** Hepatic, renal and pulmonary responses in pregnant mice and their fetuses induced by profenofos. *Journal of Union of Arab Biologists: Zoology.*; 3(A)(1995)359-386.
- [40] **Gazioano, T.A., Galea,G., and Reddy,K.S.,**Scaling up interventions for chronic disease prevention . *Lancet* ;370(2007) 1939-1946.
- [41] **Desmet, P.A. ,** The role of plant –derived drugs and herbal medicines in health care .*Drugs*; 54(1997)801-840.
- [42] **Bazo, A.P., Rodrigues, M.A.M., Sforcin, J.M., Camargo, J.L.V., Ribeiro, L.R., Salvadori, D.M.F.,** Protective action of propolis on the rat colon carcinogenesis. *Teratogenesis, Carcinogenesis and Mutagenesis.*; 22(2002)183–194.
- [43] **Banskota, A.H., Tczuka, Y., Kadota, S.,** Recent progress in pharmacological research of propolis. *Phytotherapy Research.*; 15(2001) 561-571.
- [44] **Murad, J.M., Calvi, S.A., Scares, A.M.V.C., Bankova, Y., Sforcin, J.M.,** Effects of propolis from Brazil and Bulgaria on fungicidal activity of macrophages against *Paracoccidioides brasiliensis*. *Journal of Ethnopharmacology.*; 79(2002)331-334.

- [45] **Seung Phill Choid., Kyong-Hwan Bangb., Dongho Leec.,Hyung-Kyoon Choia.,** NMR-based metabolic profiling and differentiation of ginseng roots according to cultivation ages, *Journal of Pharmaceutical and Biomedical Analysis.*;58 (2012) 19–26.
- [46] **Yun, T.K., Lee, Y.S., Lee, Y.H., Kim, S.I., Yun, H.Y.,** Anticarcinogenic effect of Panax ginseng C.A. Meyer and identification of active compounds. *Journal of Korean Medical Science.*; 16(2001)S6-S18.
- [47] **Dorval, J., Leblond, V.S., and Hontela, A., (2003):** Oxidative stress and loss of cortisol secretion in adrenocortical cells of rainbow trout (*Oncorhynchus mykiss*) exposed to in vitro to endosulfan, an organochlorine pesticide, *Aquat. Toxicol.*; 63: 229–241.
- [48] **Oakes, K.D., Mc Master, M.E., Pryce, A.C., Munkittrick, K.R., Portt, C.B., Hewitt, L.M., MacLean, D.D., and Van Der Kraak, G.J., (2003):** Oxidative stress and bioindicators of reproductive function in pulp and paper mill effluent exposed to white sucker, *Toxicol. Sci.*; 74: 51–65.
- [49] **Mechanick, J.L., (2006):** Metabolic mechanisms of stress hyperglycemia. *Journal of Parenteral and Enteral Nutrition.*; 30:157-163.
- [50] **Khani, S.,and Tayek, J.A (2001):** Cortisol increases gluconeogenesis in humans: its role in the metabolic syndrome. *Clin Sci (Lond). Dec.*; 101(6):739-47.
- [51] **Aggarwal, M., Narahariseti, S.B., Sarkar, S.N., Rao, G.S., Degen, G.H., and Malik, J.K., (2009):** Effects of subchronic coexposure to arsenic and endosulfan on the erythrocytes of broiler chickens: A biochemical study, *Arch. Environ. Contam. Toxicol.*; 56 :39–148.
- [52] **Mannaa, F., El-Shamy, Karim. A., El- Shaikh, Kamal.A., and El- Kassaby, Mahitab., (2011):** Efficacy of fish liver oil and propolis as neuroprotective agents in pilocarpine epileptic rats treated with valproate. *Pathophysiology.*;18, 287-294.
- [53] **Doyotte, C., Cossu, M.C., Jacquin, M., Babut, P., and Vasseur,S.,(1997):** Antioxidant enzymes, glutathione and lipid peroxidation as relevant biomarkers of experimental or field exposure in the gills and the digestive gland of the freshwater bivalve *Unio tumidus*, *Aquatic Toxicology* 39:93-110.
- [54] **Giulio, R.T., Benson,W.H., Sanders, B.M., and Veld, P.A.,( 1995):** Biochemical mechanisms: metabolism, adaptation and toxicity. In: Rand, G.M. (Ed.), *Fundamentals of Aquatic Toxicology. Effects, Environmental Fate and Risk Assessment.* Taylor & Francis, Washington, pp. 523–561.
- [55] **Verma, R.S.,and Srivastava, N.,(2003):** Effect of chlorpyrifos on thiobarbituric acid reactive substances, scavenging enzymes and glutathione in rat tissues, *Indian J. Biochem. Biophys.*; 40: 423-428.
- [56] **Shadnia, S., Foul addel, S., pajaoum, A., jalali, N.,and Abdollahi, M., (2005):** Evaluation of oxidative stress and genotoxicity in organophosphorous insecticide formulators, *Hum. Exp.Toxicol.*; 24: 439-445.
- [57] **Ojha, A., Yaduvanshi, S.K., and Srivastava. N., (2011):** Effect of combined exposure of commonly used organophosphate pesticides on lipid peroxidation and antioxidant enzymes in rat tissues. *Pestic. Biochem. Physiol.*; 99: 148-156.
- [58] **Ehrhart, J.,and Zeevalk, G.D.,(2001):** Hydrogen peroxide removal and glutathione mixed disulfide formation during metabolic inhibition in mesencephalic cultures. *Journal of Neurochemistry.*; 77: 1496-1507.
- [59] **Yousef, M.I.,and Salama, A.J., (2009):** Propolis protection from reproductive toxicity caused by aluminium chloride in male rats. *Food Chem. Toxic.*; 47, 1168-1175.
- [60] **Naval, M.V., Gómez-serranillos, M.P., carretero, M.E., and Villar, A.M. (2007):** Neuroprotective effect of ginseng (*Panax ginseng*) root extract on astrocytes primary culture, *Journal of Ethnopharmacology.*; 112:262-270.
- [61] **Stanic,B.,Andric,N.,Zoric, S., Grubor-Lajsic, G., and Kovacevic,R.,(2006):** Assessing pollution in the Danube Rivernear NoviSad (Serbia) using sever albiomarkers in starlet (*Acipenser ruthenus L.*). *Ecotoxicol. Environ. Saf.*; 65:395–402.
- [62] **Nagat Aly., Kawther EL-Gendy., Fatma Mahmoud., and Abdel Khalek El-Sebae., (2010):** Protective effect of vitamin C against chlorpyrifos oxidative stress in male mice. *Pesticide Biochemistry and Physiology.*; 97:7–12.
- [63] **Yu, Y., Yang, A., Zhang, H., Hu, S., and Mjtcnul, S., (2011):** Exposure in the mixture of organophosphate pesticides inducing reproductive dysfunction in the offspring. *Environ. Toxicol.*; 98:25-35.
- [64] **Bindhumol, V., Chitra, K.C., and Mathur, P.P., (2003):** Bisphenol A induces reactive oxygen species generation in the liver of male rats, *Toxicology.*; 188 :117–124.
- [65] **Verma, R.S., Mehta, A., and Srivastava, N., (2007):** In vivo chlorpyrifos induced oxidative stress: attenuation by antioxidant vitamins. *Pestic. Biochem. Physiol.*; 88: 191—196.
- [66] **Sayeed, S., Parvez, S., Pandey, B., Bin-Hafeez, R., Haque, S., and Raisuddin, N., (2003):** Oxidative stress biomarkers of exposure to deltamethrin in freshwater fish, *Channa punctatus Bloch*, *Ecotoxicol. Environ. Saf.*; 56: 295–302.
- [67] **Kono, K., Salazaronfray, F., Petersson, M., Hansson, J., Masucci, G., Wasserman, K., Nakazawa, T., Anderson, P.,and Kiessling, R., (1996):** Hydrogen peroxide secreted by tumor-derived macrophages down-modulates signal-transducing zeta molecules and inhibits tumor-specific T cell- and natural killer cell-mediated cytotoxicity. *European Journal of Immunology.*; 26: 1308-1313.
- [68] **Regoli, F., Winston, G.W. , Gorbi, S., Frenzilli, G., Nigro, M.,and Corsi, I.,(2003):** Intergrating enzymatic responses to organic chemical exposure with total oxyradical absorbing capacity and DNA damage in the European eel *Anguilla Anguilla*, *Toxicological and Environmental Chemistry.*;22:2120-2129
- [69] **Mates, J.M., (2000):** Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology.*; 153, 83–104.
- [70] **Lin, L., Liu, J., Zhang, K., and Chen, Y., (2003):** An experimental study of the effects of profenofos on antioxidant enzymes in rabbits. *Wei Sheng Yan Jiu.*; 32(5):434-5.
- [71] **Scheller, S., Wilczok, T., and Imielski, S., (1990):** Free radical scavenging by ethanolic extract of propolis. *International Journal of Radiation Biology.*; 57:461-465.
- [72] **Hyunghee L.A., Frank, J., G.B., Michung, Y.A., (2006):** Ginsenoside Rf, a component of ginseng, regulates lipoprotein metabolism through peroxisome proliferator-activated receptor  $\alpha$ , *Biochemical and Biophysical Research Communications* .;339 : 196–203.
- [73] **Halliwell, B., and Gutteridge, J.M., (1990):** Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol.*; 186:1–85.
- [74] **Mecdad,Alaa.A., Manal, H., Ahmed, B., Manal, E.A., ElHalgawy, C., Mostafa, M., and Afify, M., (2011):** A study on oxidative stress biomarkers and immunomodulatory effects of pesticides in pesticide-sprayers. *Egyptian Journal of Forensic Sciences.*; 1:93–98.
- [75] **Shah, Z.A., Gilani, R.A., Sharma, P., and Vohora, S.B.,(2005):** Cerebroprotective effect of Korean ginseng tea against global and focal models of ischemia in rats. *Journal of Ethnopharmacology.*; 101: 299–307.

- [76] **Jasprica, D., Mornar, A., Debeljak, Z., Smoteie-Bubato, A., Medfc-Saric, M., Mayer, L., Romic, Z., Bucan, K., Balog, T., Sobocanec, S., and Sverko, V., (2007):** In vivo study of propolis supplementation effects on antioxidative status and red blood cells. *J. Ethnopharmacol.*; 110:548-554.
- [77] **Cathcart, R.F., (1985):** Vitamin C: The nontoxic, nonrate-limited, antioxidant free radical scavenger. *Med. Hypotheses*, 18: 61-77.
- [78] **Rana, S.V.S., Allen, T., and Singh, R., (2002):** Inevitable glutathione, then and now. *Indian J. Exp. Biol.*; 40:706—716.
- [79] **Song, S.B., Xu, Y., and Zhou, B.S., (2006):** Effects of hexachlorobenzene on antioxidant status of liver and brain of common carp (*Cyprinus carpio*). *Chemosphere.*; 65:699–706.
- [80] **Fang, Y.Z., Yang, S., and Wu, G., (2002):** Free radicals, antioxidants, and nutrition. *Nutrition.*; 18:872–879.
- [81] **Gamal, H. Abdel Rahman., Abdel Razik, H. Farrag, Sonya, L. El Sharkawy., and Wafaa E. Abdel Aal., (2006):** Effects of Profenofos on Antioxidant Enzymes Activities and Gastric Mucosa in Rats. *JASMR.*; 1(2):125-134.
- [82] **Kavitha, P., and Venkateswara Rao, J., (2009):** Sub-lethal effects of profenofos on tissue-specific antioxidative responses in a Euryhyaline fish, *Oreochromis mossambicus*. *Ecotoxicology and Environmental Safety.*; 72:1727–1733.
- [83] **Monteiro, D.A., Almeida, J.A., Rantin, F.T., and Kalinin, A. L., (2006):** Oxidative stress biomarkers in the fresh water characid fish, *Brycon cephalus*, exposed to organophosphorus insecticide Folisuper 600 (methylparathion). *Comp. Biochem. Physiol.*; C143:141–149.
- [84] **Orsi, R.O., Funari, S.R.C., Soares, A.M.V.C., Calvi, S.A., Oliveira, S.L., Sforzin, J.M., and Bankova, V., (2000):** Immunomodulatory action of propolis on macrophage activation. *The Journal of Venomous Animals and Toxins.*; 6:205-219.
- [85] **Handy, R.D., Abd-El Samei, H.A., Bayomy, M.F., Mahran, A.M., Abdeen, A.M., and El-Elaimy, E.A., (2002):** Chronic diazinon exposure: pathologies of spleen, thymus, blood cells, and lymph nodes are modulated by dietary protein or lipid in the mouse. *Toxicology.*; 172 :13–34.
- [86] **Gokalp, O., Buyukvanli, B., Cicek, E., Ozer, M.K., Koyu, A., Altuntas, T., and Koylu, H., (2005):** The effect of diazinon on pancreatic damage and ameliorating role of vitamins E and C. *Pestic. Biochem. Physiol.*; 81:123–128.
- [87] **Kalender, S., Ogutcu, A., Uzunhisarcikli, M., Acikgoz, F., Durak, D., Ulusoy, Y., and Kalender, Y., (2005):** Diazinon induced hepatotoxicity and protective effect of vitamin E on some biochemical indices and ultrastructural changes. *Toxicology.*; 211: 197–206.
- [88] **Jintana, S., Sming, I.C., Krongtong, Y., and Thanyachai, S., (2009):** Cholinesterase activity, pesticide exposure and health impact in a population exposed to organophosphates. *mt. Arch. Occup. Environ. Health* 82: 833-842.
- [89] **Uzun, F.G., Kalender, S., Durak, D., Demir, F., and Kalender, Y., (2009):** Malathion induced testicular toxicity in male rats and the protective effect of vitamins C and E. *Food Chem. Toxicol.*; 47 : 1903–1908.
- [90] **Radhey, S., Verma, Anugya Mehta., and Nalini Srivastava., (2009):** Comparative studies on chlorpyrifos and methyl parathion induced oxidative stress in different parts of rat brain: Attenuation by antioxidant vitamins . *Pesticide Biochemistry and Physiology.*; 95: 152–158
- [91] **Abbassy, M.A., El-Nawawy, A.S., Tag El-Din, M.H., (1996):** Toxicity and residues from feeding single oral dose of Chlorpyrifos and primiphos methyl to laying hens. *Medicine Vet. Faculty, Landbouww, Rijks Uni., Gent.*; 44:273-281.
- [92] **Lukasiewicz-Hussain, A., (2010):** Role of oxidative stress in organophosphate toxicity. *Pestic. Biochem. Physiol.*; 98:145—150.
- [93] **Slotkin, T.A., (1999):** Developmental cholinotoxicants: nicotine and chlorpyrifos. *Environ Health Perspect.*; 107(Suppl.1):71—80.
- [94] **Parvez, S., and Raisuddin, S., (2006):** Copper modulates non-enzymatic antioxidants in the freshwater fish *Channa punctata* (Bloch) exposed to deltamethrin. *Chemosphere.*; 62: 1324-1332.
- [95] **Siegel, G.J., Agranoff, B.W., Albers, R.W., Fisher, S.K., and Uhler, M.D., (1999):** Basic Neurochemistry. Molecular, Cellular and Medical Aspects. 6th Edn. Lippincott-Raven Publishers, Philadelphia, New York.
- [96] **Aldridge, W.N., (1993):** Side effects of organophosphorous compounds: Delayed neurotoxicity. *Bull World Health Organization.*; 44:259-263.
- [97] **Santoni, G., Cantalamessa, F., Sagretti, O., Staffolani, M., and Piccoli, M., (1999):** Alterations of T cell distribution and functions in prenatally cypermethrin-exposed rats: Possible involvement of catecholamines. *Toxicology.*; 138: 175-187.
- [98] **Chen, C.X., Gui, Z.Y., an, Z.L., Chen, H., Ying, C., and Min, C.L., (2003):** Ginsenoside Rg1 attenuates dopamine-induced apoptosis in PC 12 cells by suppressing oxidative stress. *European Journal of Pharmacology.*; 473:1-7.
- [99] **Kim, H. K., Cheon, B. S., Kim, Y. H., Kim, S. Y., and Kim, H. P., (1999):** Effects of naturally occurring flavonoids on nitric oxide production in the macrophage cell line RAW 264.7 and their structure-activity relationships. *Biochem Pharmacol.*; 58:759-765.

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## Predicting Yarn Quality Performance Based on Fibers types and Yarn Structure

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**Abstract:** Egyptian spinning factories are faced to deterioration in their quality capabilities in the last years due to instability in cotton fiber types and quantities. This affects Quality and efficiency of knitting and weaving process as they depend on yarn properties. Instead of working with different types of Egyptian cottons the spinning factories had to process imported cotton types and polyester fibers with their trade names, for the first time, without real information's about their specifications. The aim of this work is to model the dependence of yarn quality (tenacity, evenness and imperfections) obtained within the last years at an Egyptian factory on type of cotton and polyester, twist number/factor, plying, linear density and cotton ratio of the yarn manufactured, through linear regression equations. Models concerning the different cotton fibers, blends of cotton and polyester and both the two groups are obtained. Linear regression equations relating the dependence of yarn properties obtained within the last five years at an Egyptian factory on material and yarn structures was determined, this will enable the factory to plan and improve the yarn quality level. Cotton type, yarn count and twist have the higher effect on all the properties studied also the yarn tensile strength and its variation depend on most of the factors studied. Cotton type Giza 86 give the best yarn properties followed by Giza 90 and Greece cotton fibers respectively of all yarns. A fifty percent of polyester fibers in blended yarns improved the tensile properties beside to evenness.

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**Keywords:** Yarn Quality; Performance; Fiber; Yarn Structure

### 1.Introduction

In the present competitive age, quality of textile product is the most desirable factor at purchase counter for the consumer. It is also helpful in keeping the cost of production within satisfactory level [1]. Therefore, quality is one of the key factors for the Egyptian textile industry's success. Egyptian textile manufacturers need to maintain a high standard of quality [2]. Globally, cotton growing and processing has remained one of the profitable industries, which employs large sections of the populations and also earns foreign exchange [3].

Yarn quality is an essential concept defined by customer which requests the satisfaction of several properties simultaneously [4]. The quality characteristics belong to the input and the output of this industry where as reference values belong to the processing itself. The Optimum operating conditions depends on fibers' characteristic and working conditions [2]. The main cotton fiber properties are length, fineness, strength and elongation. In addition to these properties, maturity and number of convolutions are also important. Furthermore, color, the amount of trash and the humidity that the cotton fiber retains are properties which affect the cotton quality. These properties should be known in order to produce yarns and fabrics of appropriately high quality [5-6]. Among fiber properties, strength has the greatest effect on the yarn unevenness and higher

fiber strength lead to a better yarn evenness value of the Egyptian cotton [7].

Yarn strength, which is considered to be the most important property of spun yarns, is largely influenced by the tenacity, length, length uniformity, short fiber content and fineness (micronaire reading) of the constituent cotton fibers [5]. Also a high correlation between fiber and yarn elongation exist [6, 8], also the amount of short fibers increases the yarn strength, hairiness and number of thin and thick places. More neps in the fibers results more neps in yarns [8]. Quality of final yarn is largely influenced (up to 80%) by the specification of raw cotton. However, yarn manufacturing technology determines the level effect of various fiber properties [9].

The homogeneity in raw material has an effect on the uniformity of the end product [1]. Yarn structure (yarn count, yarn twist), unevenness properties (CV% values of unevenness, thin and thick places, neps, hairiness) and physical and mechanical properties (strength, elongation) are the main yarn properties [6]. Many of the yarn quality characteristics are highly correlated with each other, a variation in one characteristic will adversely affect many other yarn properties. Yarn linear density is highly correlated with other yarn quality characteristics [3]. The effect of yarn linear density is significant on yarn properties. As the yarn linear density increase evenness, the number of thin place, the number of thick place and neps values increase,

hairiness, diameter, tenacity and elongation values decrease. In carded yarns, increase of twist coefficient increases the evenness, tenacity and elongation values and decreases hairiness [10].

For a given count the yarn strength is high if the evenness is low because the number of weak places is low as well. There is a linear relationship between yarn strength and yarn elongation. The tenacity depends on the twist and the fiber strength [8].

The tensile properties of spun yarn are of the most important parameters for assessment of yarn quality. The tensile properties decide the performance of post spinning operations; warping, weaving and knitting and the properties of the final textile structure; hence its accurate technical evaluation carries much importance in industrial applications [11]. The breaking load of spun yarn is one of the most important properties in determining the yarn quality, since it is directly affect the winding, weaving and knitting efficiency, so the properties of yarn are very important in determining their possible applications [12, 13].

Nevertheless, customers usually require much more quality criterion [4]. The yarn tensile properties depend upon the properties of its constituent fibers, the arrangements of these fibers within the yarn (i.e. on the yarn structure), and the mass distribution of yarn along its length. The structure is primarily decided by the yarn formation mechanism and the process parameters. For a yarn spun from a given fiber, an increase in tenacity is associated with an increase in breaking extension, that is, the tenacity and extension are expected to respond together in a similar fashion. Imperfections usually increase as the yarn becomes finer [14].

Egyptian cotton is the world's finest cotton because it has some noble characteristics apart from other natural fibers. The length of the fiber makes it possible to make the finest of yarns without sacrificing the strength of the yarn. The strength of the fiber makes yarn and fabric more solid and more resistant to stress. Its ability to absorb liquids gives fabrics made of Egyptian cotton deeper, brighter and more resistant colors. Its softness feels like nothing else in the world [7].

The process of converting fibers into yarn is complex, and requires many investigations and new technical & technological solutions. The quality of the spun yarn can be significantly improved, while using equally raw material, by a suitable selection of the spinning system and the type of the spinning machine used [12]. There is no perfect fiber. All fibers have well, far and poor characteristics so blending is the technique to combine fibers which emphasizes the good qualities and minimizes poor qualities of the fibers [15].

Blending of different types of fibers is normally preferred for enhancing the performance as well as esthetic values of fabric. Blend yarns of natural and man-made fibers have the particular advantages such as comfort of wear and easy care parameters, this permit the manufacturer to meet marketing conditions. The blending effect depends on resultant blend components [16].

Blending cotton/polyester fibers is common practice in the textile industry. However, it is a critical problem in fiber blending technology to choose appropriate types of fibers and blend ratios depending on the final product. The properties of the blended yarns cannot merely be explained in terms of the proportions of the different constituent fibers in the blends [17]. The blended yarns have breaking strengths lower than those expected from the summation of the proportioned constituent fiber component strengths [11].

The weaving technology prefers ply yarns than single yarns since unevenness, imperfection, hairiness and protruding fibers are lower, and for that the sizing operation is eliminated. Unevenness of polyester/cotton two-ply yarn increases as single and ply twist factors increase. The increase of ply twist reduces the variation in hairiness [18].

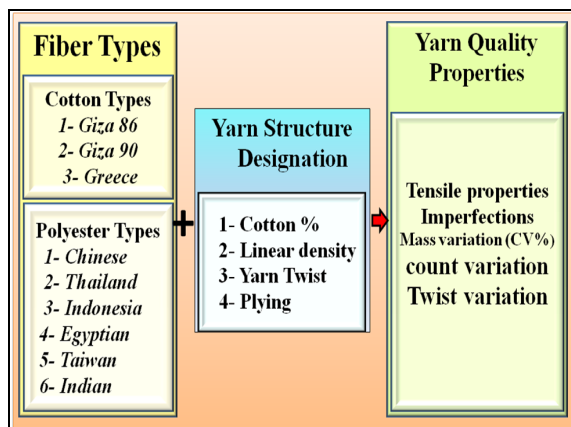
As yarn strength is the principle component yarn quality and the most important index of spinning quality, predicting yarn strength is very important from a technological point of view.

Many mathematical models have been used to understand and predict the complex relationships between fiber parameters and yarn characteristics, and substantial research has been done to determine methods of predicting yarn properties [19].

Up-to-date information of the fiber properties, every year, is needed to guide the textile industry in attaining their maximum utilization, since fiber properties changes due to hybrid of natural fibers or progress in synthetic. This will result in minimum end breakage, maximum yarn quality and production rates in the spinning organizations. The production of fine yarns and high quality ready-made garments from Egyptian cotton lint can be attained [20].

## 2. Experimental Work

The materials utilized in this study consist of carded yarns produced in an Egyptian spinning mill within the five successive years 2007-2011. In the present study, 3 types of cotton fibers, and six staple polyester types produced by 6 different companies of different country were used to produce one hundred and six yarns on a ring spinning machines (cotton and blended yarns of cotton /polyester 50:50%) different in linear density, twist characteristics, and single and two plied as shown in Figure 1.



**Figure 1.** The Experimental Studied Factors and Yarn Quality Properties

The lint cotton varieties Giza 86, Giza 90, and Greece cotton were collected from the running stock of the mills. Physical characteristics of cotton fibers i.e. spun length, uniformity ratio, fineness, strength and trash were determined according to standard methods. The Shirley analyzer was applied for the determination of fiber and trash content, the Sutter Web Comb Sorter was used to determine fiber length properties, the Presley for fiber strength, Micronaire for fineness and for maturity. Fiber properties of the studied cotton varieties are presented in table 1. The data recorded in **Table 1** reveal that the cotton varieties involved in this study differ widely with respect to their fiber properties.

**Table 1.** Characteristics of Cotton Types

Cotton Types		Cotton Types		
		Giza 86	Giza 90	Greece
Parameter	Value			
Trash	Fibers%	96.6	90.6	96.3
	Trash%	3.4	9.4	3.7
Fiber Length	Effective Length	34.8	28.8	31.5
	Evenness %	75.8	68.3	73.3
	Short Fiber%	19.6	26.8	23.6
Tenacity	gm/tex	26.1	21.1	17.9
	Mature Guide	83.9	73.7	82.5
Fineness	Microgram/inch	4.33	3.3	3.9
	Denier	1.56	1.2	1.38
Neps/gm		12	20	25

The cotton fibers consisted of three types (Giza 86, Giza 90 and Greece) are coded according to their tenacity from one to three in order. The polyester fibers consisted of six types (Chinese, Thailand, Indonesian, Egyptian, Taiwan and Indian), they are coded from one to six respectively according to their length and extension.

The fibers are processed on this system using standard mill procedures, adjustments and practices on a traditional ring spinning mill. The yarn linear density varies from 16 to 40 (Ne), the twist multiplier for single yarns varies from 3.45- 4.6 and the twist multiplier for two plied yarns from 2.7- 4.2. The qualities of yarns were evaluated for their physical and mechanical properties.

All the yarn variants were tested at the factory laboratory according to the standard methods in order to assess such yarn quality properties as breaking force, R.K.M., elongation at break, variation in strength, variation in linear density, twist variation, unevenness (CV%), and yarn imperfection (thick, thin places and neps).

### 3. Results and Discussion

A relation for predicting the yarn quality obtained from an Egyptian spinning mill within the last five years was obtained based on the fiber types and yarn structure, the factors under study consist of six factors; four quantitative (cotton percent, yarn linear density, yarn twist and the number of folds) and two qualitative (cotton type and polyester type). Linear regression equations for the mean property of every count and yarn type within one year were employed. Three groups of regression equations were determined for every property the first group for all the fiber types (106 yarns), the second for the blended yarns (58 yarns) while the third for the hundred percent cotton only (48 yarns).

The results of the regression equations were assessed at significance levels of  $P$  value  $\leq 0, 05$  and significant value  $\leq 0, 01$ .

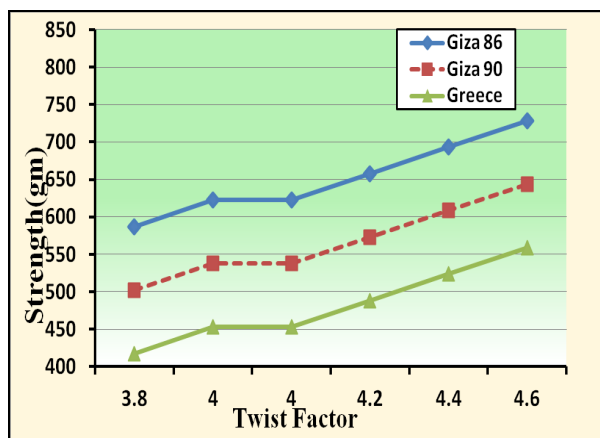
In the following discussion, the results of yarn quality properties of the regression equations are divided into three main units which are tensile properties, imperfection (the number of thin-thick places, and neps) and unevenness included variation of mass CV%, linear density, twist and strength.

#### The Yarn Tensile Properties

Tensile properties including yarn breaking strength, Yarn Elongation and Rupture per kilometer (RKM) were tested on the principle of constant rate of extension (CRE). Table (1) demonstrates the equations of tensile properties for the three yarn groups. From table (1) the R Square values are from 0.66-0.93 which confirms a strong relation between the factors under study and yarn strength and RKM. The cotton type and blending ratio dominate all the equations and depends on cotton fiber tensile properties. Giza 86 give better results followed by Giza 90 than Greece fibers. From Figures (2, 3) the distinct influence of cotton type on the yarn strength can be seen.

**Table 2.** The Equations of Yarn Tensile Properties

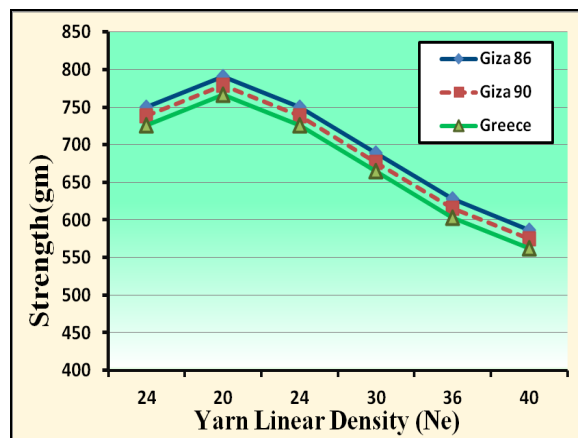
	Yarn Strength			RKM			Elongation%		
	All	Blend	Cotton	All	Blend	Cotton	All	Blend	Cotton
Intercept	1071	755.96	1575.4	30.46	26.36	25.5	10.0	9.6	7.83
Cotton Type	-84.7	-49.23	-123.3	-2.83	-1.91	-3.68			
Polyester Type							0.16	1.7	
Cotton %	-176.4			-6.74			-4.6		
Ne		8.46	-10.22	0.053	0.098	-0.04	0.06	0.04	
Twist Factor	176.9	236.09					1.48	1.07	
Twist No./Meter	-1.28	-1.68	-0.81	-0.003	-0.007		-0.008	-0.008	-0.002
Ply	110.9	66.6	108.1	0.98	1.56		-0.41		
R Square	0.86	0.93	0.86	0.74	0.66	0.89	0.84	0.72	0.13

**Figure 2.** Relation between Yarn Strength of Cotton Types and Twist Factor

No doubt that plying yarns is increasing yarn strength and RKM. Increasing the twist factor, within the working range, increase yarn strength and tenacity as presented in Figure (2), however increasing the number of twists give an opposite results. This can indicate a quadratic relation since the numbers of twist consist of the twist factor multiplied by the root of the English count.

The coarse yarn would always be stronger than a thinner yarn made from similar fibers and under similar processing conditions however this is only obtained in the equations for the cotton yarns group only as shown in Figure (3) and not the case when blended yarns are involved in the relation. When determining the regressions with the yarn count only as a factor the sign for the yarn count is negative for the three group of relations, the former result can be due to correlation between some factors in case of the blended yarns.

The values of R Square for elongation equations are 0.84 and 0.72 in case of first and second groups respectively which indicate a good

**Figure 3** Relation between Yarn Strength of Cotton Types and Yarn Linear Density

predicted relation, while it is only 0.13 for the third relation. This is due to the small difference in percent elongation for the different cotton types. The yarn elongation at break increases with the increase in the twist factor, within the factory working range, which relates to the angle of twist helix the surface fibers have in a yarn. The blended yarn elongation depends on the polyester type as shown in Figure (4); its coefficient is higher for the second group since no cotton yarns enter in the relation.

In contrast to yarn strength and RKM, the obtained relation demonstrate that the elongation at break is smaller for plied yarns than yarn elongation of single yarns, this is obtained since the ply twist direction is the same as the single twist. Adding polyester fibers in yarns is improving the yarn elongation. The influence of polyester type on the value of the tensile and RKM is unimportant but it was significant in yarn elongation.

#### The Yarn Imperfection Properties

Yarn faults in the shape of thin places, thick places, and neps which are called the imperfections



are decisive on the external appearance of yarns and the products obtained from them. A yarn is only as strong as its weakest spot. The Uster evenness tester was used to provide a considerable amount of information about the imperfections. The equations

of imperfections by regression analysis were obtained in table (3). From table (3) the dependency of the imperfection on cotton types which were selected was significant. The yarns spun of Greece cotton fibers have the highest imperfections.

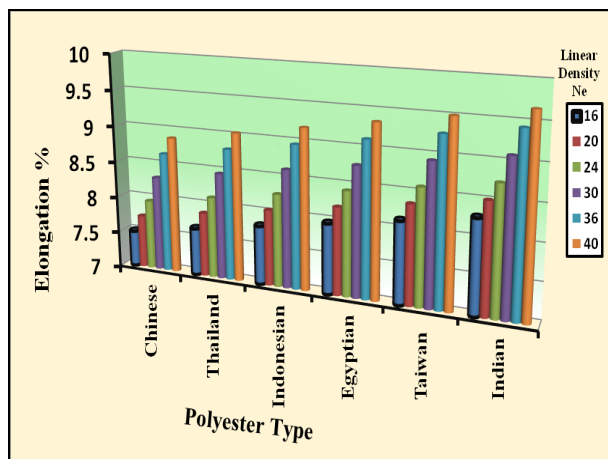


Figure 4. Effect of Polyester type and yarn Density on Yarn Elongation

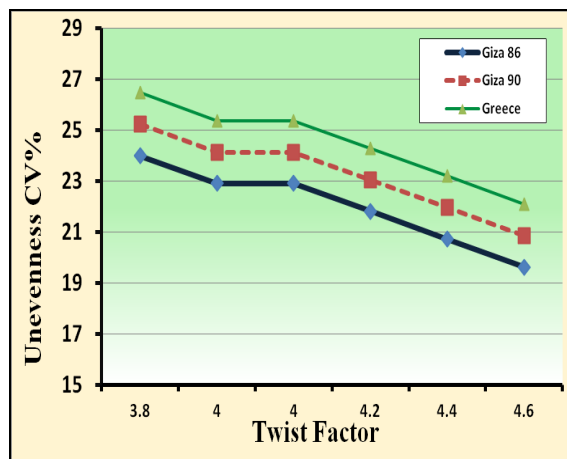


Figure 5. Effect of Twist Factor and Cotton Types on Yarn Unevenness

Table 3. The Equations of Imperfection Properties

	Thin Places			Thick Places			Neps		
	All	Blend	Cotton	All	Blend	Cotton	All	Blend	Cotton
Intercept	-81.0	0.0	-72.7	0.0	0.0	-831	-1315	0.0	-986.6
CottonType	16.67	20.93	10.3	181.1	180	131.4	163.7	215.1	107.17
Polyester Type	-4.5								
Cotton %	-38.3						-267.6		
Ne	1.26					6.64			
Twist Factor		-51.96		-269.3	-385.5	198.9		-584.1	
Twist No./Meter	0.1	0.25	0.09	1.49	2.1		2.16	3.16	1.47
Ply				-101.8		-242.8			
R Square	0.43	0.63	0.38	0.87	0.91	0.61	0.5	0.81	0.32

In general, the imperfection level is found to increase with the increase in yarn twist number, while the higher twist factor led to the lower yarn imperfections except thick places in cotton yarns. Since the twist factor has different sign in the regression equation for cotton yarns and blended yarns, regression equations for the effect of twist factors only demonstrate the same sign. From that the reverse of the twist sign can be due to the different type of polyester, Further studies has to be taken to attain the real causes. The plied yarns were found to have the lowest thick places, while blended yarns showed lowest number of thin places and neps.

#### The Yarn Unevenness Properties

Evenness is the most important quality aspect of a yarn, because variations in yarn properties often result in unwanted patterning in fabrics made from

such yarns that affects the quality of the resultant fabrics. They also lead to increased ends down during spinning and subsequent processing. Obviously the higher the CV% value, the more irregular the yarn is. Mass variations in a yarn CV% were measured by using the Uster evenness tester. The average values of CV% for yarn linear density, twist and tensile variation were calculated. The relation between unevenness properties and factors under study were modeled in table (4). It shows the regression coefficients of variables and R square

Equations in table (4) indicates that Greece cotton fibers give the highest variations in mass and tensile for produced yarns followed by Giza 90 and G86 cotton fibers in order. This result is shown in Figure (5). The results showed that there were a highly negative correlation between twist number and

both linear density and tensile variations. Whereas, the correlation between twist number and yarn evenness CV % were a positive direction. Variation in tensile and yarn linear density is increasing as the number of twist factor increases. The worst results in mass and linear variations were obtained with yarn fineness.

**Table 4.**The Equations of Variation Properties

	CV% Unevenness			CV% Linear density			CV% Twist			CV% Tensile		
	All	Blend	Cotton	All	Blend	Cotton	All	Blend	Cotton	All	Blend	Cotton
Intercept	20.3	31.1	8.54	0.0	0.0	2.82	0.0	4.23	0.0	0.0	-15.2	0.0
CottonType	1.24	1.03	1.46							0.91	1.32	1.01
Polyester Type												
Cotton %	-1.27											
Ne	-0.24	-0.22				-0.071		0.08	-0.04	0.21	0.12	0.33
Twist Factor	-5.46	-8.08		0.827	0.75		0.62			2.67	5.93	3.61
Twist No./Meter	0.03	0.03	0.007	-0.002	-0.003			-0.004	0.004	-0.012	-0.008	-0.02
Ply			-3.26					-1.06	0.98	-2.9	-2.25	-2.67
R Square	0.63	0.6	0.6	0.94	0.93	0.2	0.92	0.3	0.96	0.95	0.32	0.96

The quality of factory data is represented by the proposed regressions to a great extent. Only twenty percent of these regressions attain an R-square lesser than 0.5, Sixty six percent of them are in the 100% cotton group. This can be due to the lack in readjustments of machines under different fiber types. In the main time it was better to carry this study taking into consideration material properties and factory working conditions, as the quality of yarns depends on them. But this was not applied due to lack in factory data base.

#### Conclusion

The proposed regressions describe the factory yarn properties results to a great extent. The yarn quality levels obtained in the studied factory is very low and is in the range of 75%to 95% Uster statistics. This is due to the fact that the factory has no the cultures of working with dynamic variations in cotton or polyester fibers. In addition no real information's about their specification is available when they are purchased.

Although the data base of the factory is uncompleted the effects of the studied factors coincide with the known practices. Predicting the yarn quality obtained from an Egyptian spinning mill within the last five years based on the fiber types and yarn structure was obtained, this will enable the factory to plan any required yarn properties after that. Applying the results of this study the factory can improve the yarn quality level.

Most of the significant regression equations obtained have an R-square more than 0.5. The best

The effect of plying on unevenness results are not similar in all type of yarns observed in this study. As expected, plying yarns improved the evenness of tensile properties. Whereas plying cotton yarns increased the variation in twist.

results are obtained when working every fiber group separately. The worst results were obtained in the 100% cotton group, this can be due to the constant working conditions for the different fibers, a condition which was difficult to confirm due to factory policies.

Cotton type, yarn count and twist are the factors which have the higher effect on all the properties studied about eighty percent of all the coefficients. Due to that the factory manager has to adjust the processing so that they correspond to these three factors. In the main time the yarn tensile strength and its variation is the properties that demonstrate its dependence on most of the factors studied, about forty percent of the confidants are related to it.

As expected the quality obtained from Greece fibers is the worst, so if using such a fiber or a similar one the factory has to mix him with an Egyptian fiber for better quality and maintaining a reasonable expenses. Also the yarn obtained from 50:50 cotton polyester blend give better yarn properties as expected.

Further improvement in the factory yarn quality if the manager adjusts the working conditions based on the clear understanding of the mechanism of yarn formation and the way the fiber parameters interact for each type of fibers.

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**References**

- 1- Ahmed, M. S, Javed, I., Ahmad, M., Naeem, H.M., and Sarwar, M. (1992). **Application of Statistical Quality Control in Yarn Spinning**, Pak. J. Agri. Sci., Vol. 29, No.(1): pp 25-30
- 2- Azzam, H. A. and Mohamed, S.T. (2005). **Adapting and Tuning Quality Management in Spinning Industry**, AUTEX Research Journal, Vol. 5, No (4): , December pp 246-258
- 3- Mwasiagi, J. I. (2011). **Use of SOM to Study Cotton Growing and Spinning**, Self Organizing Maps - Applications and Novel Algorithm Design, pp 577-600
- 4- Souid, H., and Cheikhrouhou, M. (2011). **Slup Yarn Quality Optimization by Using Desirability Function and Neural Networks**, Journal of Applied Science 11(17) pp: 3204-3208,
- 5- Hussein, K. M., Hassan, A. H. and Kamal, M. M. (2010). **The Multiplicative Analytic Hierarchy Process (MIAHP) as a Quality Criterion Determining the Technological Value of the Egyptian Cotton Varieties**, American Journal of Plant Sciences, 1: 106-112
- 6- Karapinar, B. O. and Erdem, N. (2003). **Comparison of Quality Characteristics of Yarns Spun from Aegean Cotton Fibres and Their Mixtures with South-East Anatolian Cotton Fibres**, Fibers and Textiles in Eastern Europe, Vol. 11, No.(4) (43), pp: 26-29
- 7- Mahmood, N., Tusief, M. Q., Arshad, M. and Azeem, M. (2010). **Physico- Chemical Study of Egyptian and Common Wealth of Independent States(CIS) Long Staple Cotton and Spinning Potential Evaluation at Ring and Compact Spinning Systems under Some Mechanical Variables**, Pakistan Journal of Science ,Vol. 62, No.(4): , pp 2-9
- 8- Furter, R. (2009). **Uster Think Quality, Standards from Fiber to Fabric, Physical properties of spun yarns**, Uster Technologies AG , 3rd Edition: June SE 586, pp 1-36
- 9- Majumdar, A., Majumdar, P. K. and Sarkar, B. (2005). **Determination of the Technological Value of Cotton Fiber: A Comparative Study of the Traditional and Multiple-Criteria Decision-Making Approaches**, AUTEX Research Journal, Vol. 5, No(2), June pp: 71-80
- 10- Altas, S., and Hüseyin Kadoğlu, H. (2012). **Comparison of Conventional Ring, Mechanical Compact and Pneumatic Compact Yarn Spinning Systems**, Journal of Engineered Fibers and Fabrics, Volume 7, Issue (1): , pp 87-100
- 11- Das, B. R. (2010). **Tensile Behaviour of Spun Yarns under Static State**, Journal of Engineered Fibers and Fabrics, Volume 5, Issue (1): , pp 1-9
- 12- Jackowska-Strumiłło, L., Cyniak, D., Czekalski, J. (2007). **and Jackowski, T., Quality of Cotton Yarns Spun Using Ring, Compact and Rotor-Spinning Machines**, Fibers and Textiles in Eastern Europe, Vol. 15, No.(1): (60), pp 24-30
- 13- Elshakankery, M.H., El-Sayed, M.A.M., and Sanad, S. H. (2008). **A Method of Predicting the Breaking Load of Egyptian Extra Long Staple Cotton by Using Neural Networks**, Journal of Applied Sciences Research, 4(11), pp: 1380-1386
- 14- Chattopadhyay, R., and Sinha, S. K. (2007). **A Study on Spinning Limits and Yarn Properties with Progressive Change in Yarn Count in Friction Spinning**, AUTEX Research Journal, Vol. 7, No(1): , March pp 1-8
- 15- Bhardwaj, S., and Juneja, S. (2012). **Performance of Jute Viscose/Polyester and Cotton Blended: Yarns for Apparel Use**, Stud Home Com Sci, 6(1), pp: 33-38
- 16- Mahmood, N., Jamil, N.A., Arshad, M., Tusief M.Q. and Iftikhar, M. (2009). **Interaction Study of Polyester and Multi Bleached Cotton Blends for the Tensile Properties of Rotor Spun Melanage Yarn**, Pak. J. Agri. Sci., Vol. 46(1) pp: 46-50
- 17- Baykal, P. D., and Erol, O. B. R. (2006). **Prediction of Strength and Elongation Properties of Cotton/Polyester-Blended OE Rotor Yarns**, Fibers and Textiles in Eastern Europe, Vol. 14, No.(1): (55), pp 18-21
- 18- Palaniswamy, K., and Mohamed, P. (2006). **Effect of the Single-Yarn Twist and Ply to Single-Yarn Twist Ratio on the Hairiness and Abrasion Resistance of Cotton Two-Ply Yarn**, AUTEX Research Journal, Vol. 6, No(2): , June pp 59-71
- 19- Nurwaha, D., and Wang, X. H. (2012). **Using Intelligent Control Systems to Predict Textile Yarn Quality**, Fibers and Textiles in Eastern Europe, 20, 1(90), pp: 23-27
- 20- Mohamed, E. A. M., Sanad, S. H., and Arafa, A. S. (2009). **Spinning Performance of ELS Egyptian Cotton**, African Journal of Agricultural Research, Vol. 4 (11), November pp: 1276-1283.

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## Comparative Study Assessing the Effect of Tigecycline and Moxifloxacin in Prevention of *Acinetobacter baumannii* Biofilm

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**Abstract: Background & Aim of work:** In recent years there has been an increase in life-threatening infections caused by *Acinetobacter baumannii* with multiple antibiotic resistance, especially, in case of biofilm formation. This study aimed at assessing the rate of multidrug resistance (MDR) among *A. baumannii* isolates, incidence of biofilm formation and evaluating the role of, recently produced medications from 2 different antibiotic classes; tigecycline (glycylcyclines) and moxifloxacin (fluroquinolones), in prevention of *Acinetobacter* biofilm formation.

**Methods:** The current investigation was carried out on 30 strains of *A. baumannii* isolated from different samples at King Khalid University Hospital. Strains were identified and characterized for their antibiotic sensitivity. The MICs tests were conducted to all yields for tigecyclin and moxifloxacin antibiotics. The frequency of biofilm formation and strength was determined by modified microtitre plate method. Finally, prevention of biofilm formation was done using 1X MIC, 2X MIC and 0.5X MIC concentrations of tigecyclin and moxifloxacin.

**Results:** All *A. baumannii* isolates were found to be MDR strains, resistance to tested antibiotic discs were found to be 100% in 23.3% of the tested strains, 90% of them were biofilm formers. MIC to tigecyclin was found to be 100% sensitive to all yields with MIC<sub>90</sub> and MIC<sub>50</sub> equal to 0.5 µg/ml and 0.25 µg/ml respectively. However, 83.3 % of the strains were resistant to moxifloxacin with MIC<sub>90</sub> and MIC<sub>50</sub> equal to 32 µg/ml. In testing their ability in avoidance of biofilm formation, unexpectedly, the reduction in biofilm formation were more noticed in the tested concentrations of moxifloxacin with high significance compared with controls in the 3 concentrations tested with ( $p < 0.001$ ). While, tigecycline gave a gradual reduction; double MIC, MIC, 0.5 MIC concentrations showed the significance of ( $p < 0.001$ ,  $< 0.01$  and  $< 0.05$ ) respectively.

**Conclusion and Recommendations:** Most *A. baumannii* isolates are MDR isolates with high tendency of biofilm formation, tigecyclin was the most effective tested antibiotic used on the planktonic cells while its effect on biofilm was exceeded by moxifloxacin. Further investigation is needed to understand the molecular basis of such an interesting finding.

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**Keywords:** *Acinetobacter baumannii*, Biofilm, tigecyclin, moxifloxacin

### 1. Introduction

*Acinetobacter* species are primarily associated with nosocomial infections in severely ill patients, particularly with ventilator associated pneumonia, wound infection and bacteraemia (Cisneros *et al.*, 1996). Multidrug-resistant *Acinetobacter baumannii* is a rapidly emerging pathogen in the health care setting, where it causes infections that include bacteremia, pneumonia, meningitis, urinary tract infection, and wound infection. The organism's ability to survive under a wide range of environmental conditions and to persist for extended periods of time on surfaces make it a frequent cause of outbreaks of infection and an endemic, health care-associated pathogen (Fournier *et al.*, 2006 and Jawad *et al.*, 1996).

The management of *Acinetobacter baumannii* infections can be difficult, due to the increasing

number of isolates exhibiting resistance to multiple classes of antibacterial agents (Fagon *et al.*, 1993 and Cisneros *et al.*, 1996). Because multidrug-resistant *Acinetobacter* infection usually occurs in severely ill patients in the ICU, the associated crude mortality rate is high, ranging from 26% to 68% (Seifert *et al.*, 1996, Sunenshine *et al.*, 2007 and Kwon, 2007).

The potential ability of *A. baumannii* to form biofilms could explain its outstanding antibiotic resistance and survival properties. This possibility is supported by a very limited number of publications which showed that a clinical isolate of this bacterium is able to attach to and form biofilm structures on glass surfaces (Vidal *et al.*, 1996, Vidal *et al.*, 1997 and Epsinal *et al.*, 2012). Bacterial biofilms, arrangements in which the cells are morphologically, metabolically and physiologically different from their planktonic

counterparts (Stoodley *et al.*, 2002), have been found on the surface of medical devices such as intubation tubes, catheters, artificial heart valves, water lines and cleaning instruments (Donlan & Costerton, 2002).

Moreover, many studies have tried newly produced antibiotics in treatment of MDR strains of *Acinetobacter* species. Tigecycline, a relatively new glycolcycline agent, has been found to have bacteriostatic activity against *Acinetobacter* species (Pachon-Ibanez *et al.*, 2004 and Seifert *et al.*, 2006). *Acinetobacter baumannii* pathogen has been shown to be susceptible to tigecycline in large-scale microbiological studies (Petersen *et al.*, 2002, Bradford *et al.*, 2005 and Betriu *et al.*, 2006). Tigecycline confers its activity by reversibly binding to the 30S subunit of the ribosome. It acts by blocking the incorporation of transfer RNA into the A site of the ribosome, thus inhibiting protein synthesis (Garrison *et al.*, 2005). In addition, newer fluoroquinolones, such as moxifloxacin, have shown increased activity against *Acinetobacter baumannii* *in vitro* in comparison with older agents such as ciprofloxacin (Vila *et al.*, 2002 and Wisplinghoff *et al.*, 2003). Moxifloxacin is a broad spectrum 8-methoxyquinolone which interacts preferentially with DNA gyrase in Gram-negative organisms (Zhanel *et al.*, 1999).

Therefore, the current study aimed at assessing the rate of multidrug resistance (MDR) among *A.baumannii* isolates, incidence of biofilm formation and evaluating the role of, recently produced medications from 2 different antibiotic classes; tigecycline (glycolcyclines) and moxifloxacin (fluoroquinolones), in prevention of *Acinetobacter* biofilm formation.

## 2. Subjects and Methods:

### 1. Bacterial isolates:

In the current study, isolates of *A.baumannii* were obtained from King Khalid Hospital Microbiology Laboratory, KSU, Riyadh, KSA, from various clinical samples. Clinical data were collected including; sex, age, site of sample as well as departments involved. Further processing of the isolates was performed at microbiology laboratories at College of pharmacy, King Saud University and Faculty of Medicine, Fayoum University.

### 2. Confirming the identity of *Acinetobacter* strains:

Verifying the identity of the yields was conducted by the various conventional methods including; culture on selective media, colony morphology, microscopic examination and oxidase test. Oxidase-negative Gram-negative Bacilli were further identified by Microbact (12A) Gram-negative identification system (Oxoid, Basingstoke, UK) to separate *A. baumannii*.

### 3. Antimicrobial susceptibility tests:

Disc diffusion test: Antibiotic sensitivity of the isolates was determined using the Kirby-Bauer antibiotic testing (KB testing or disk diffusion

antibiotic sensitivity testing) on Mueller-Hinton agar as recommended by the Clinical and Laboratory Standards Institute (CLSI, previously called NCCLS) (2006 guidelines). Susceptibility to the following antimicrobial agents was performed: amoxicillin + clavulanic acid, ampicilline, colistine, cefotaxime, ceftriaxone, cefepime, ciprofloxacin, meropenem, sulfamethoxazole, piperacillin + tazobactam, ceftazidime, imipenem, tetracycline, gentamicin, and amikacin.

MIC test: In addition, MICs of tigecyclin and moxifloxacin antibiotics were determined for each isolate using 0.5 McFarland standard. Serial two-fold dilutions of the two antibiotics were distributed in 96 microtiter plate. The inoculum suspension and standardization is done according to Clinical and Laboratory Standards Institute. The bacterial inoculum is then inoculated into the wells and incubated at 37°C overnight. MICs were then determined. Isolates were considered susceptible if the MIC of tigecyclin was  $\leq 2$   $\mu\text{g/mL}$  and resistant if the MIC was  $\geq 8$   $\mu\text{g/mL}$ , while, for moxifloxacin susceptible if  $\leq 1$   $\mu\text{g/mL}$  and resistant if the MIC was  $\geq 2$   $\mu\text{g/mL}$ . *Escherichia coli* (strain ATCC 25922) was used as the control strain for disc susceptibility testing and MICs. (CLSI, 2006)

### 4- Biofilm formation

Biofilm formation was determined as follows. Overnight cultures were diluted to 0.5 McFarland using Brain Heart Infusion broth supplemented with 3% sucrose as a growth medium (Oxoid, Madrid, Spain), deposited in 96-well plates and incubated at 37°C for 48 h without shaking. Biofilm was stained with 0.4% Crystal Violet (w/v) and quantified at 590 nm after solubilization with 95% ethanol. The experiment was performed in triplicates. OD590 values for each well were subtracted from those of the blank, which only contained Brain Heart Infusion broth without inoculum (Stepanovic *et al.*, 2000, Stepanovic *et al.*, 2007 and Yanti *et al.*, 2009). *A. baumannii* ATCC 19606 strain was employed as the positive control.

Biofilm calculation: The optical density (ODs) of each strain was obtained by the arithmetic mean of the absorbance of three wells and this value was compared with the mean absorbance of negative controls (ODnc). The following classification was used for the determination of biofilm formation: no biofilm production (ODs=ODnc), weak biofilm production (ODnc<ODs $\leq$ 2.ODnc), moderate biofilm production (2.ODnc<ODs $\leq$ 4.ODnc) and strong biofilm production (4ODnc<ODs). (Rodrigues *et al.*, 2010)

### 5. Assess the effect of Tigecycline and Moxifloxacin antibiotics on biofilm prevention:

The strongest 10 biofilm former isolates were selected to evaluate biofilm prevention. Using tigecyclin and moxifloxacin antibiotics in 2X MIC, 1X MIC, and ½X MIC concentrations, they were distributed in 96 microtiter plate, each in triplicates.

The bacterial inoculum was then inoculated into the wells and incubated at 37°C for 2 and 4 days. Degree of biofilm formation was detected using XTT/menadione reagent, incubated in the dark for 2 hrs at 37 °C and quantified at 490 nm. The experiment was performed in duplicates to detect settling cells quantity. Furthermore, the strongest two biofilm formers were subjected for further investigation to assess biofilm prevention per time period. XTT assay were done at 1, 2, 4, 6, 48 and 96 hours and planktonic cells were observed by inverted microscope at same time period (Yanti *et al.*, 2009).

### 6- Statistical analysis:

Data were expressed as means  $\pm$  S.D. For multi-variable comparisons, one-way ANOVA was conducted, followed by Bonferroni testing using the GRAPHADA INSTAT (ISI Software) computer program. Differences were considered significant at  $P < 0.05$ .

### 3. Results:

The current study included thirty *Acinetobacter baumannii* isolates, collected from different clinical specimens in King Khalid Hospital, Microbiology Laboratory, Rhiyadh, SA. The patients included in the study their ages ranged between 1-94 years, with a mean of  $43.9 \pm 28.9$  years. They were 20 males representing 66.7% of the cases and 10 females representing 33.3 %. Most of the isolates were collected from different ICU wards within the hospital being 13 cases representing 43.3%. Moreover, the most frequent specimens were from wound infections representing 56.7% (17 specimens) of the cases followed by bone infections 13.3% (4 specimens), catheter borne infections 10% and then by ear infection, respiratory tract infection and urinary tract infection 6.6% each.

Twenty three percent of the total strains were resistant to 100% antibiotics, 33.33% of the strains were resistant to 75 – 90% antibiotics and 66.66% were resistant to 50 – 75% as shown in table 2. Susceptibility distribution is shown in table 1.

**Table 1.** Antimicrobial susceptibility results (percent) of *Acinetobacter baumannii* isolates

Antibiotics	Frequency of resistance	Intermediate	Sensitive
Amikacin	15 (50%)	-	15 (50%)
Gentamicin	12 (40%)	1 (3.3%)	17 (56.7%)
Amoxicillin + clavulanic acid	23 (76.7%)	-	7 (23.3%)
Piperacillin +Tazobactam	21 (70%)	3 (10%)	6 (20%)
Ampicillin	30 (100%)	-	-
Ceftazidime	20 (66.7%)	-	10 (33.3%)
Ceftriaxone	20 (66.7%)	2 (6.66%)	8 (26.66%)
Cefotaxime	23 (76.7%)	1 (3.3%)	6 (20%)
Cefepime	23 (76.7%)	-	7 (23.3%)
Imipenem	22 (73.3%)	-	8 (26.7%)
Meropenem	27 (90%)	-	3 (10%)
Ciprofloxacin	25 (83.3%)	-	5 (16.7%)
Sulfamethoxazole	14 (46.7%)	-	16 (53.3%)
Colistin	7 (23%)	-	23 (76.66%)
Tetracycline	16 (53.33%)	5 (16.66%)	9 (30%)

**Table 2.** Percentage of resistant strains against the used antibiotics

Antibiotics	Number of isolates showing resistance	Percent
AK, GM, AMC, PTZ, AMP, CAZ CRO, CTX, FEP, IMP, MEP, CIP, SXT, COL, TCN	7	23.33
GM, AMC, PTZ, AMP, CAZ, CRO, CTX, FEP, MEP, CIP, COL, TCN	10	33.33
AMP, AMC, CRO, CTX, FEP, MEP, CIP, TCN	20	66.66
AMP, FEP, MEP, CIP	23	76.66

AK- Amikacin, GM- Gentamicin, AMC- Amoxicillin + clavulanic acid, PTZ- Piperacillin +Tazobactam, AMP- Ampicillin, CAZ- Ceftazidime, CRO- Ceftriaxone, CTX- Cefotaxime, FEP- Cefepime, IMP- Imipenem, MEP- Meropenem, CIP- Ciprofloxacin, SXT- Sulfamethoxazole, COL- Colistin, TCN- Tetracycline.

One hundred percent of the isolates were sensitive to tigecycline with MIC<sub>90</sub> and MIC<sub>50</sub> equal to 0.5 µg/ml and 0.25µg/ml respectively. However, 83.3 % of the

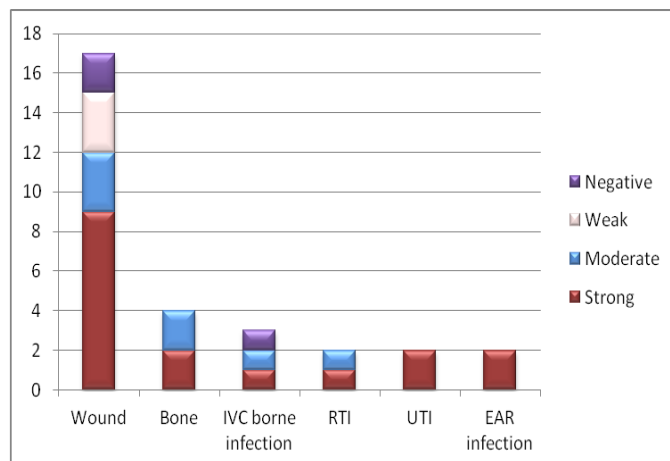
strains were resistant to moxifloxacin with MIC<sub>90</sub> and MIC<sub>50</sub> equal to 32 µg/ml as shown in table 3.

**Table 3.** MICs of Tigecycline and Moxifloxacin in the tested isolates

	MIC <sub>90</sub>	MIC <sub>50</sub>	Maximum MIC	Minimum MIC	MIC mean
Tigecycline	0.5	0.25	0.5	0.06	0.34
Moxifloxacin	32	32	64	0.125	28

In the current study, **90%** of strains were biofilm former (**56.7%** strong, **23.3%** moderate, **10%** weak biofilm former). There were no significant difference ( $p$  value > 0.05) found related to the site of sample collection shown their distribution in figure 1.

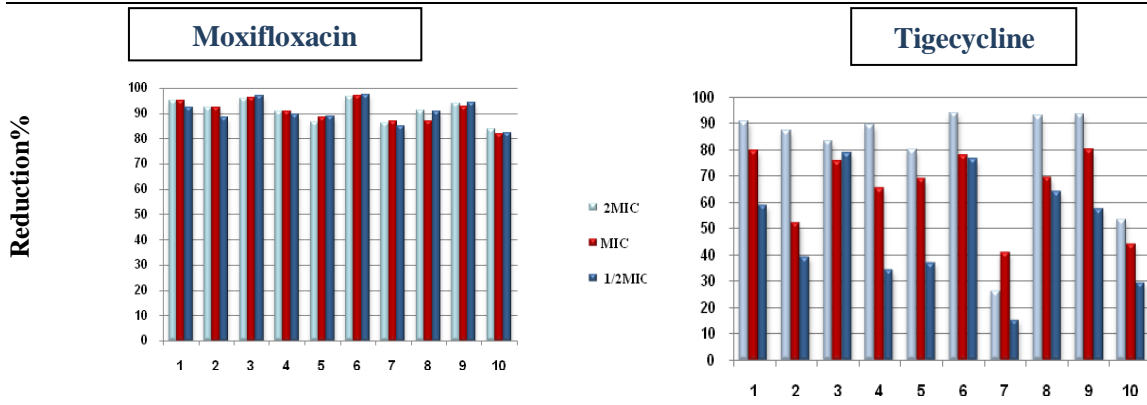
Moreover, there were no relationship detected between the degree of biofilm formation and ability of the organisms to show multidrug resistance, in fact, negative and weak biofilm formers showed the highest resistance as shown in table 4.

**Figure 1:** Distribution of isolates biofilm strength among different sample sites**Table 4.** Relationship between biofilm strength and antimicrobial resistance among the tested yields

Antibiotics	Resistance				
	Biofilm strong isolates (n=17)	Biofilm intermediate isolates (n=7)	Biofilm weak isolates (n=3)	Biofilm negative isolates (n=3)	Resistance of all isolates (n=30)
Amikacin	4 (23.5%)	5 (71.4%)	3 (100%)	3 (100%)	15 (50%)
Gentamicin	4 (23.5%)	4 (57.1%)	3 (100%)	1 (33.33%)	12 (40%)
Amoxicillin + clavulanic acid	15 (88.24%)	5 (71.4%)	2 (66.66%)	1 (33.33%)	23 (76%)
Piperacillin + Tazobactam	12 (70.56%)	5 (71.4%)	2 (66.66%)	2 (66.66%)	21 (70%)
Ampicillin	17 (100%)	7 (100%)	2 (100%)	3 (100%)	30 (100%)
Ceftazidime	11 (64.7%)	4 (57.12%)	3 (100%)	2 (66.66%)	20 (66.66%)
Ceftriaxone	11 (64.7%)	4 (57.12%)	3 (100%)	2 (66.66%)	20 (66.7%)
Cefotaxime	13 (76.47%)	5 (71.4%)	3 (100%)	2 (66.66%)	23 (76.7%)
Cefepime	12 (70.56%)	5 (71.4%)	3 (100%)	3 (100%)	23 (76.7%)
Imipenem	10 (58.82%)	7 (100%)	3 (100%)	2 (66.66%)	22 (73.3%)
Meropenem	15 (88.24%)	6 (85.71%)	3 (100%)	3 (100%)	27 (90%)
Ciprofloxacin	14 (82.35%)	5 (71.4%)	3 (100%)	3 (100%)	25 (83.3%)
Sulfamethoxazole	8 (47.05%)	2 (28.57%)	2 (66.66%)	2 (66.66%)	14 (46.7%)
Colistin	3 (17.64%)	3 (42.85%)	1 (33.33%)	0	7 (23%)
Tetracycline	10 (58.82%)	3 (42.85%)	2 (66.66%)	1 (66.66%)	16 (53.33%)

In testing the ability of tigecycline and moxifloxacin in prevention of biofilm formation, unexpectedly, the reduction in biofilm formation were more noticed in the tested concentrations of moxifloxacin with high significance ( $p <$

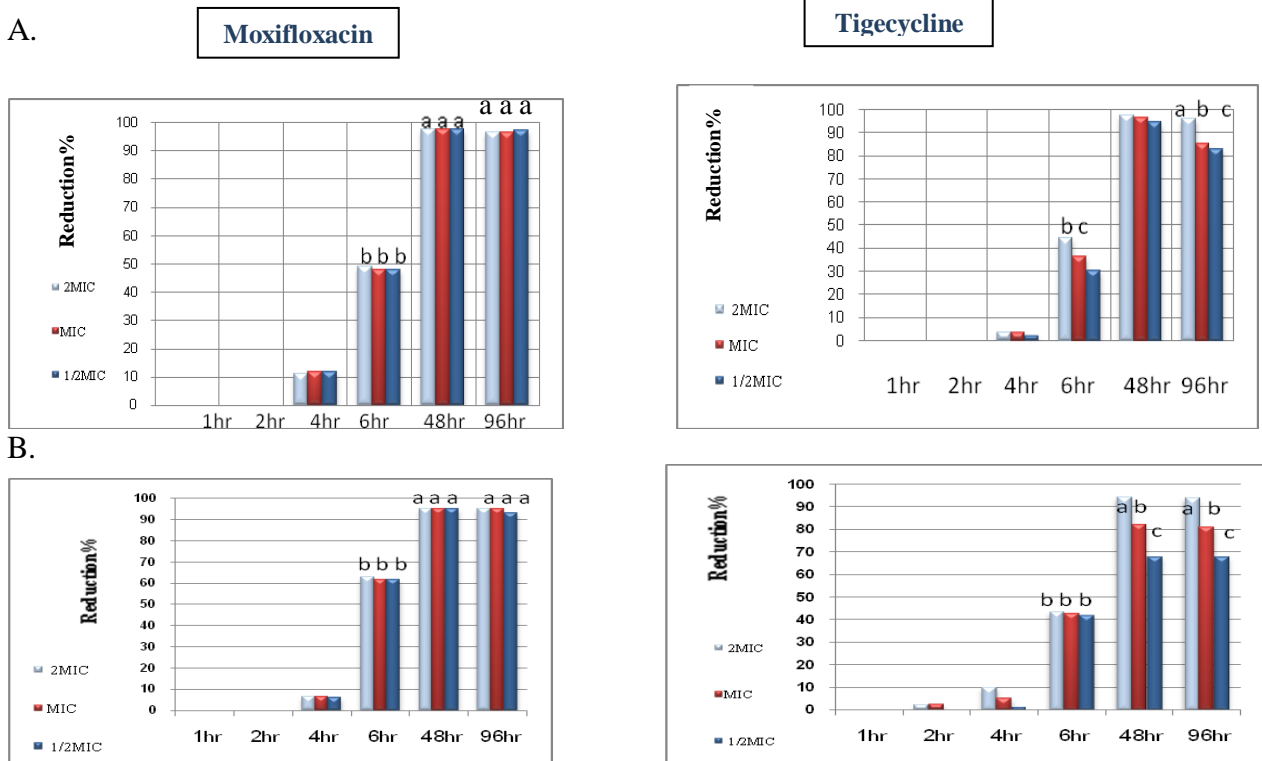
0.001) compared with controls in the 3 concentrations tested with. While, tigecycline gave a gradual reduction; double MIC, MIC, 0.5 MIC concentrations showed the significance of ( $p < 0.001$ ;  $< 0.01$  and  $< 0.05$ ) respectively as shown in Figure 2, 3.



**Figure 2.** The effect of moxifloxacin and tigecycline in the 3 concentrations tested among the ten isolates investigated

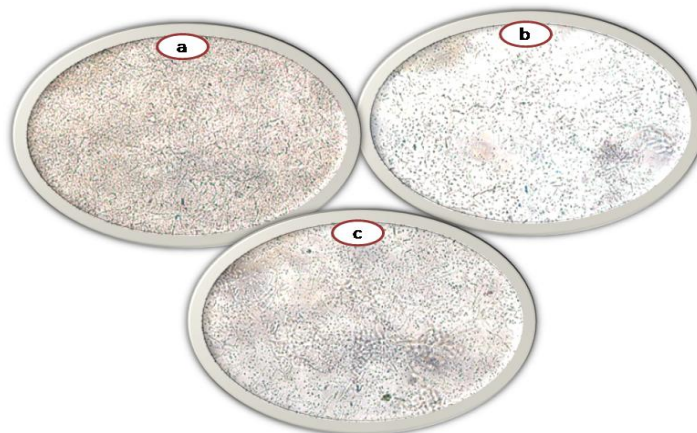


**Figure 3:** The minimum inhibitory concentration shown of several isolates indicating the higher sensitivity of tigecyclin over moxifloxacin in the tested isolates



**Figure 4:** Reduction Charts of two isolates at different time periods per hour. The values are expressed in % of biofilm reduction . <sup>a</sup>P < 0.001 <sup>b</sup>P < 0.01, <sup>c</sup>P < 0.05 compared to control strains.





**Figure 5: *A. baumannii* under inverted microscope X 385 a- control strain after 48 hours , b- tigecycline effect on the planktonic cells in 2XMIC concentration, c- moxifloxacin effect on the planktonic cells in 2XMIC concentration.**

#### 4. Discussion:

*A. baumannii* is an opportunistic pathogen that is particularly successful at colonizing and persisting in the hospital environment, able to resist desiccation (Jawad *et al.*, 1996, Cappelli *et al.* and 2003, Dima *et al.*, 2006) and survive on inanimate surfaces for months (Jawad *et al.*, 1997). It is among the most common causes of device-related nosocomial infection which result when the organism is able to resist physical and chemical disinfection, often by forming a biofilm (Thomas *et al.*, 2008).

The age of the patients included in the present study ranged between 1 and 94 years, with a mean of  $43.9 \pm 28.9$  years. They were 20 males representing 66.7% of the cases and 10 females representing 33.3%. These findings were in agreement with Gaber *et al.* (2010) who reported that no specific age for acinetobacter infected patients and found that the mean age was  $43.6 \pm 21.2$  years (ranging between 10 and 90 years) and the higher incidence of infection was observed in males (19/30) than in females (11/30). Also Husni *et al.* (1999) found that the mean age of the patients was 50 (ranging between 21 and 84 years), and 79% of them were males. Most of the patients included were inpatients in different ICU wards being 13 cases representing 43.3%. Similar finding was reached by Fournier and Richet (2006), they found 29 out of 44 cases were isolated from ICU wards. Gaber *et al.* (2010) reported that critically ill patient in ICUs at a higher risk of nosocomial infections due to prolonged ICU stay, underlying chronic diseases, disruption of barriers (endotracheal intubation, tracheostomy, urinary catheterization and CVC) and prolonged use of antimicrobial therapy.

Moreover, the most frequent specimens were collected from wound infections representing 56.7% of the cases followed by bone infections 13.3%, catheter borne infections 10% and then ear infection, respiratory tract infection and urinary tract infection

6.6% each. In agreement with present study Iregbu *et al.* (2002), Joshi *et al.* (2003) and Joshi *et al.* (2006) reported higher incidence of acinetobacter spp. was found in wound infections but this followed by urinary tract infections. In contrast to our findings, Lee *et al.* (2004) found that the most frequently isolated yields were from respiratory secretions representing 57%. Other sites of isolation included wound (19%), blood borne (4%), urine (3%), and bile (3%). These difference in rate and type of infections may be due to variation of infection control protocols in ICUs in some researches or isolation of acinetobacter spp. from different wards of the hospitals not only from ICUs in other researches.

In the current study, 30 *A. baumannii* strains were isolated, 100% of which were MDR isolates. Disc diffusion method was employed to assess the sensitivity of the isolates to different antibiotics, 23.3% were found to be resistant to 100% of the tested antibiotics. MICs by microdilution method were conducted to assess the effect of tigecycline and moxifloxacin on all yields. 100% of the isolates were sensitive to tigecycline with MIC<sub>90</sub> and MIC<sub>50</sub> equal to 0.5 µg/ml and 0.25 µg/ml respectively. However, 83.3% of the strains were resistant to moxifloxacin with MIC<sub>90</sub> and MIC<sub>50</sub> equal to 32 µg/ml. Comparable results were found by Henwood *et al.* (2002) who found that more than 90% of the isolates were sensitive to tigecyclin by the broth microdilution method, with MIC<sub>90</sub> and MIC<sub>50</sub> equal to 2 µg/ml and 0.5-1 µg/ml respectively. Moreover, the study conducted by Soussy *et al.* (2003) was in agreement with the effect of moxifloxacin on *A. baumannii* in the present study, they found that moxifloxacin displayed poor activity against *A. baumannii* whereas it was more active against *A. lwoffii* and other *Acinetobacter* species (MICs between 0.032mg/L and 0.5mg/L).

In the current study, 90% of strains were biofilm formers (56.6% strong, 23.3% moderate, 10% weak

biofilm former). These findings were comparable with the results of Wroblewska *et al.* (2008) who found that 100% of strains were biofilm formers in their study, however, only 12% were strong biofilm formers, 47% moderate and 41% were weak. Moreover, there was no correlation found between degree of biofilm formation and antibiotic resistance (Table 4). Several studies had similar conclusion, they found that weak biofilm formers were presenting high antibiotic resistance pattern among their isolates (Rodriguez-Ban *et al.*, 2008 and Epsinal *et al.*, 2012). Nevertheless, some others correlated between antibiotic resistance and strong biofilm formation (Wendt *et al.*, 1997, Lee *et al.*, 2008; and Rao *et al.*, 2008).

In addition, there were no relationship detected between the degree of biofilm formation and site of sample collection (Figure 1). These results agreed with Wroblewska *et al.* (2008) who found that *A. baumannii* site of isolation had no significant effect on their ability to produce biofilm.

In testing the avoidance of biofilm formation, unexpectedly, the reduction in biofilm formation was more noticed in the tested concentrations of moxifloxacin with high significance compared with controls in the 3 concentrations tested with  $p$  value of  $< 0.001$ . Pompilio *et al.* (2010) found comparable results; biofilm production was significantly lower ( $p < 0.001$ ) in the presence of 0.036X and 0.066X MIC on a different tested organism. No statistically significant differences were observed between inhibition levels caused by 0.066X and 0.036 X MIC exposures for tested strains. Biofilm inhibition by moxifloxacin may be explained through different mechanisms; inhibition of the synthesis or expression of adhesins on the bacterial cell surface or modifying the bacterial shape in such a way that interferes with the ability of the micro-organisms to approach host cell-surface receptors (Lorian & Ernst, 1987; Lorian *et al.*, 1989). Fluoroquinolones were found in many studies to reduce the virulence of some bacteria (Gram-negative bacilli, in particular) by inducing an elongation of the cell soma along its longitudinal axis, a phenomenon also known as filamentation. These morphologically altered cells generally show reduced pathogenicity in terms of lower levels of adhesion, altered susceptibility to phagocytosis and decreased release of bacterial enzymes (Labro *et al.*, 1987; Chen *et al.*, 2005). In addition, Drago *et al.* (2005) observed that moxifloxacin subMICs (0.125 $\times$  and 0.06 $\times$  MIC) induced filamentation in a remarkable portion of *Klebsiella pneumoniae* in an animal experimental model of pulmonary infection.

However, some previous studies on *E. coli* found the effect of fluoroquinolones was limited at concentrations equal to or not less than 0.125 $\times$  MIC on biofilm prevention (Baskin *et al.*, 2002; Wojnicz & Jankowski, 2007).

On the other hand, tigecycline gave a gradual reduction on biofilm formed by the isolates; double MIC, MIC, 0.5 MIC concentrations showed significant growth reduction with values of  $p < 0.001$ ;  $< 0.01$  and  $< 0.05$  respectively. These results were consistent with the work of Maestre *et al.* (2012); who found that biofilm reduction was highly significant on their tested strains, however, there were no difference in biofilm reduction ( $p < 0.001$ ) for the 2 tested concentrations (0.25 $\times$  MIC, and 0.5 $\times$  MIC) of tigecycline.

#### Conclusion and Recommendations:

All thirty *Acinetobacter* isolates were multidrug resistant organisms, 90% of which were biofilm formers, and 57% of the tested strains were strong biofilm formers. Tigecycline was the most effective tested antibiotic against *Acinetobacter* yields (100% sensitivity), while, moxifloxacin showed much lower activity (83.3% were resistant). However, though tigecyclin was effective against biofilm formation, its effect was exceeded by moxifloxacin in the tested concentrations in prevention of biofilm formation. Further investigation is needed to understand the molecular basis of such an interesting finding.

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#### 5. References

- Baskin, H., Doğan, Y., Bahar, I. H. & Yuluğ, N. (2002). Effect of subminimal inhibitory concentrations of three fluoroquinolones on adherence of uropathogenic strains of *Escherichia coli*. *Int J Antimicrob Agents* 19, 79–82.
- Betriu C, Rodriguez-Avial I, Gomez M *et al.* (2006). Antimicrobial activity of tigecycline against clinical isolates from Spanish medical centers. Second multicenter study. *Diagn Microbiol Infect Dis*; 56:437–44.
- Bradford PA, Weaver-Sands DT, Petersen PJ (2005). *In vitro* activity of tigecycline against isolates from patients enrolled in phase 3 clinical trials of treatment for complicated skin and skin-structure infections and complicated intra-abdominal infections. *Clin Infect Dis*; 41 Suppl 5: S315–32.
- Cappelli, G., L. Sereni, M. G. Scialoja, M. Morselli, S. Perrone, A. Ciuffreda, M. Bellesia, P. Inguaggiato, A. Albertazzi, and C. Tetta. (2003). Effects of biofilm

- formation on haemodialysis monitor disinfection. *Nephrol. Dial. Transplant.* 18:2105–2111.
- Chen, K., Sun, G. W., Chua, K. L. & Gan, Y. H. (2005). Modified virulence of antibiotic-induced *Burkholderia pseudomallei* filaments. *Antimicrob Agents Chemother* 49:1002–1009.
- Cisneros JM, Reyes MJ, Pachon J *et al.* (1996). Bacteremia due to *Acinetobacter baumannii*: epidemiology, clinical findings, and prognostic features. *Clin Infect Dis*; 22: 1026–32.
- Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 16th informational supplement, M100-S16. Wayne, PA: CLSI, 2006.
- Dima, S., E. I. Kritsotakis, M. Roubelaki, S. Metalidis, A. Karabinis, N. Maguina, F. Klouva, S. Levidiotou, E. Zakyntinos, J. Kioumis, and A. Gikas. (2007). Device-associated nosocomial infection rates in intensive care units in Greece. *Infect. Control Hosp. Epidemiol.* 28:602–605.
- Drago, L., De Vecchi, E., Nicola, L. & Gismondo, M. R. (2005). Evaluation of antibacterial *in vitro* activity of moxifloxacin and its effects on pulmonary clearance of *Klebsiella pneumoniae* in an animal experimental model. *Arzneimittelforschung* 55, 473–477.
- Donlan, R. M. & Costerton, J. W. (2002). Biofilms: survival mechanisms of clinically relevant microorganisms. *J Clin Microbiol Rev* 15: 167–193.
- Epsinal P., Marti S. and Vila (2012). Effect of biofilm formation on the survival of *Acinetobacter baumannii* on dry surfaces. *Journal of Hospital Infection*, 80 :56-60
- Fagon JY, Chastre J, Hance AJ *et al.* (1993). Nosocomial pneumonia in ventilated patients: a cohort study evaluating attributable mortality and hospital stay. *Am J Med*; 94: 281–8.
- Fournier PE & Richet H (2006). The epidemiology and control of *Acinetobacter baumannii* in health care facilities. *Clin Infect Dis* 1;42 (5):692-9.
- Gaber SN, Iskander RM, Ouda NH and Bassyouni RH (2010). Demographic features and antimicrobial susceptibility of *Acinetobacter* species isolated from Intensive Care Units. *Egyptian J. of Medical Microbiology.* 19(5): 281-290.
- Garrison MW, Neumiller JJ, Setter SM (2005). Tigecycline: an investigational glycolipopeptide antimicrobial with activity against resistant Gram-positive organisms. *Clin Ther*; 27: 12–22.
- Henwood CJ, Gtward T, Warner M, James D, *et al.* (2002). Antibiotic resistance among clinical isolates of acinetobacter in UK, and *in vitro* evaluation of tigecyclin (GAR-936). *Journal of Antimicrobial Chemotherapy*, 49: 479-487.
- Husni RN, Goldstein L S., Arroliga A C., Geraldine S. Hall G S, Fatica C., Stoller J K., and Gordon S M (1999). Risk Factors for an Outbreak of Multi-Drug-Resistant *Acinetobacter* Nosocomial Pneumonia Among Intubated Patients. *Chest*; 115:1378-1382.
- Iregbu K, Ogunsola F and Odughemi T (2002). Infection caused by *Acinetobacter* spp. and their susceptibility to 14 antibiotics in Lagos University Teaching hospital, Lagos West Afr. *J. Med.* 21: 226-229.
- Jawad, A., J. Heritage, A. M. Snelling, D. M. Gascoyne-Binzi, and P. M. Hawkey (1996). Influence of relative humidity and suspending menstrua on survival of *Acinetobacter* spp. on dry surfaces. *J. Clin. Microbiol.*, 34:2881–2887.
- Jawad, A., H. Seifert, A. M. Snelling, J. Heritage, and Hawkey P. M. (1998). Survival of *Acinetobacter baumannii* on dry surfaces: comparison of outbreak and sporadic isolates. *J. Clin. Microbiol.*, 36:1938–1941.
- Joshi S, Litake G, Satpute M *et al.* (2006). Clinical and Demographic features of infection caused by *Acinetobacter* spp. *Ind. J. Med. Sci.*, 60: 351-360.
- Joshi S, Litake G, Niphadkar K *et al.* (2002). Multidrug resistant *A. baumannii* isolated from a teaching hospital. *J. Infect. Chemother*; 9:187-190.
- Kramer, A., I. Schwebke, and Kampf G. (2006). How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect. Dis.*, 6:130.
- Kwon KT, Oh WS, Song JH, *et al.* (2007). Impact of imipenem resistance on mortality in patients with *Acinetobacter* bacteraemia. *J Antimicrob Chemother*; 59:525–30.
- Labro, M. T., Pochet, I., Babin-Chevaye, C. & Hakim, J. (1987). Effect of ceftriaxone-induced alterations of bacteria on neutrophil bactericidal function. *J Antimicrob Chemother* 20: 857–321`869.
- Lee SO, Kim NJ, Choi SH, Kim TH, Chung JW, Woo JH, Ryu J, and Kim YS (2004). Risk Factors for Acquisition of Imipenem-Resistant *Acinetobacter baumannii*: a Case-Control Study. *Antimicrobial Agents and Chemotherapy*, Jan, p. 224–228.
- Lee H, Koh Y, Kim J, *et al.* (2008). Capacity of multidrug-resistant clinical isolates of *Acinetobacter baumannii* to form biofilm and adhere to epithelial cell surfaces. *Clin Microbiol Infect*; 14:49e54.
- Lorian, V. & Ernst, J. (1987). Effects of antibiotic on bacterial structure and their pathogenicity. *Pathol Biol.*, 35:1370–1376.
- Lorian, V., Ernst, J. & Amaral, L. (1989). The post-antibiotic effect defined by bacterial morphology. *J Antimicrob Chemother* 23: 485–491.
- Maestre J R., Aguilar L., Mateo M., Giménez M J., Méndez M L., Alou L., Granizo J J., and Prieto J (2012). *In vitro* interference of tigecycline at subinhibitory concentrations on biofilm development by *Enterococcus faecalis*. *J. Antimicrob. Chemother.* 10:1093.
- Pachon-Ibanez ME, Jimenez-Mejias ME, Pichardo C, Llanos AC, Pachon J (2004). Activity of tigecycline (GAR-936) against *Acinetobacter baumannii* strains, including those resistant to imipenem. *Antimicrob Agents Chemother*; 48:4479–81.
- Petersen PJ, Bradford PA, Weiss WJ *et al* (2002). *In vitro* and *in vivo* activities of tigecycline (GAR-936), daptomycin, and comparative antimicrobial agents against glycopeptide-intermediate *Staphylococcus aureus* and other resistant Gram-positive pathogens. *Antimicrob Agents Chemother*; 46: 2595–601.
- Pompilio A., Catavittello C., Picciani C., Confalone P., Piccolomini R, Savini V., Fiscarelli E., Antonio D D. and Bonaventura G Di, (2010). Subinhibitory concentrations of moxifloxacin decrease adhesion and biofilm formation of *Stenotrophomonas maltophilia* from cystic fibrosis. *Journal of Medical Microbiology*, 59:76–81.)
- Rao R, Karthika R, Singh S, *et al.* (2008). Correlation between biofilm production and multiple drug resistance

- in imipenem resistant clinical isolates of *Acinetobacter baumannii*. Indian J Med Microbiol; 4:333e337.
- Rodriguez-Ban˜o J, Marti S, Soto S, *et al.* (2008). Biofilm formation in *Acinetobacter baumannii*: associated features and clinical implications. Clin Microbiol Infect;14:276e278.
- Rodrigues LB ; Santos LR; Tagliari VZ; Rizzo NN; Trenhago G; Oliveira AP; Goetz F and Nascimento VP (2010) . Quantification of biofilm production on polystyrene by *Listeria*, *Escherichia coli* and *Staphylococcus aureus* isolated from a poultry slaughterhouse. Brazilian Journal of Microbiology ,41: 1082-1085.
- Seifert H, Strate A, Pulverer G. (1995). Nosocomial bacteremia due to *Acinetobacter baumannii*: clinical features, epidemiology, and predictors of mortality. Medicine (Baltimore); 74:340–9.
- Seifert H, Stefanik D, Wisplinghoff H, (2006). Comparative *in vitro* activities of tigecycline and 11 other antimicrobial agents against 215 epidemiologically defined multidrug-resistant *Acinetobacter baumannii* isolates. J Antimicrob Chemother; 58:1099–100.
- Soussy CJ, Nguyen J, Goldstein F, Dabernat H, Andermont A, *et al.* (2003). *In vitro* antibacterial activity of moxifloxacin against hospital isolates: a multicenter study. European Society of Clinical Microbiology and Infectious Diseases; 9:997-1005.
- Stepanovic, S.; Vukovic, D.; Dakic, I.; Savic, B.; Svabic-Vlahovic, M.(2000). A modified microtiter-plate test for quantification of staphylococcal biofilm formation. J. Microbiol. Methods 40, 175–179.
- Stepanovic, S.; Vukovic, D.; Hola,V.; Bonaventura, G.; Djukic, S.; Irkovic, I.C.; Ruzicka, F. (2007). Quantification of biofilm in microtiter plates: overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS 71 (5): 687–690.
- Stoodley, P., Sauer, K., Davies, D. G. & Costerton, J. W. (2002). Biofilms as complex differentiated communities. Annu Rev Microbiol., 56: 187–209.
- Sunenshine RH, Wright MO, Maragakis LL, *et al.* (2007). Multidrug-resistant *Acinetobacter* infection mortality rate and length of hospitalization. Emerg Infect Dis.; 13:97–103.
- Thomas W. Loehfelm, Nicole R. Luke and Anthony A (2008). Identification and Characterization of an *Acinetobacter baumannii* Biofilm-Associated Protein. J. Bacteriol., 190(3):1036.
- Vidal, R., Dominguez, M., Urrutia, H., Bello, H., Garcia, A., Gonzalez, G. & Zemelman, R. (1997). Effect of imipenem and sulbactam on sessile cells of *Acinetobacter baumannii* growing in biofilm. Microbios, 91:79–87.
- Vidal, R., Dominguez, M., Urrutia, H., Bello, H., Gonzalez, G., Garcia, A. & Zemelman, R. (1996). Biofilm formation by *Acinetobacter baumannii*. Microbios, 86:49–58.
- Vila, J., Ribera, A., Marco, F. *et al.* (2002). Activity of clinafloxacin, compared with six other quinolones, against *Acinetobacter baumannii* clinical isolates. Journal of Antimicrobial Chemotherapy, 49: 471–7.
- Wendt C, Dietze B, Dietz E, Ruden H (1997). Survival of *Acinetobacter baumannii* on dry surfaces. J Clin Microbiol.; 35:1394e1397
- Wisplinghoff, H., Decker, M., Haefs, C. *et al.* (2003). Mutations in *gyrA* and *parC* associated with resistance to fluoroquinolones in epidemiologically defined clinical strains of *Acinetobacter baumannii*. Journal of Antimicrobial Chemotherapy, 51: 177–80.
- Wojnicz, D. & Jankowski, S. (2007). Effects of subinhibitory concentrations of amikacin and ciprofloxacin on the hydrophobicity and adherence to epithelial cells of uropathogenic *Escherichia coli* strains. Int J Antimicrob Agents, 29: 700–704.
- Wroblewska M M., -Grzelak A S, Marchel H, Lucza M & Alex Sivan (2008). Biofilm production by clinical strains of *Acinetobacter baumannii* isolated from patients hospitalized in two tertiary care hospitals. FEMS Immunol Med Microbiol., 53: 140–144
- Yanti, Rukayadi Y., Lee KH, and Hwang JK (2009). Activity of panduratin A isolated from *Kaempferia pandurata* Roxb. against multi-species oral biofilms *in vitro*. Journal of oral Science, Vol. 51, No(1);, pp.87-95.
- Zhanel GC, Walkty A, Vercaigne L *et al.* (1999).The new fluoroquinolones: a critical review. Can J Infect Dis; 10 (3): 207–38.

**The comparative study of Internet purchase behavior: British students and international students in London**

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**Abstract:** This paper investigates the impact of demographic factors including age, gender, household income level, education level and nationality on Internet shopping behavior of British students and International students resident in London. In fact, this study attempts to look at the association exists between the variables involved, by tapping the responses of 84 respondents from postgraduate British and overseas students living in London. In this research, we conducted a questionnaire survey based on a model called AIDA (Attention, Interest, Desire and Action) to explain consumer behavior of online shopping. SPSS17.0 was used in order to analyzing the data. Our results suggest that demographic characteristics of age, gender, and education level, as well as household income level and nationality have no effects on student's online shopping behavior. The main findings of the current study demonstrated that the viewpoints of British students and international students in terms of limitations and advantages of shopping online are different. The first group considered "Wider variety of goods to choose" and "Disappearing of shopping website" as the advantages and limitations of Internet shopping respectively. The second group, International students resident in London, perceived "Time Saving" and "Credit card security issue" as the main advantages and limitations of online shopping respectively.

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**Key Words:** Internet, online shopping behavior, International students, British students, demographic factors

**Introduction**

The emergent of Internet has introduced new opportunities for human beings life that did not previously exist. One aspect of it, online shopping, is becoming increasingly popular. For instance, In the UK 8.1% of total retail sales in April 2010 were online sales which are greatly expanded over recent years (<http://www.statistics.gov.uk>). A report explains that 79% of UK internet users used the internet to order goods or services in 2010 which is higher than any other European country (<http://consumers.ofcom.org.uk>). Thus, the need to study the behavior of consumers is seriously recognized. To date, from the perspectives of consumer demographics, online behavior has been widely examined (O'Keefe et al. 2000, Chau et al. 2002, Park et al. 2004, Brown et al. 2003, Stafford et al. 2004, Korgaonkar et al. 2004, Park and Jun 2003, Li et al. 1999)

The principal purpose of this study is to assess the demographic factors associated with students' shopping behavior. A secondary, precursor aim is to identify whether British students and International students behave differently through online shopping. Finally, the advantages and limitations of online purchase from both group perspectives. The rest of the paper is organized as follows: First, we review

consumer factors that influence online shopping behavior in Section 2. Then, we explain consumer behavior of online shopping by the help of AIDA model, and present research methodology in Section 3. In Section 4, a conclusion summarizes the findings and the results.

**Shopping behavior**

Consumer behaviour can be defined as the decision processes to search, select, purchase, use, and dispose of goods and services, in satisfaction of individuals' needs and wants. In this regard, factors affected on internet shopping have been broadly examined. As a result, early research showed that across different studies some consumer factors is known to have consistent effects, while others have mixed or even contradictory impacts on Internet shopping. For instance, from the perspectives of consumer demographic profile, online shopping behavior has been broadly examined (Park et al. 2004, Li et al. 1999, Stafford et al. 2004, Brown et al. 2003, Hyokjin, et al. 2002, Donthu and Garcia, 1999, Chau et al. 2002, Doolin, et al. 2005, O'Keefe et al. 2000, Park and Jun 2003, Korgaonkar et al. 2004, Teo, 2001).

**AIDA Model**

The classical model of AIDA (Attention - Interest - Desire - Action) was first promoted by E.K. Strong in 1925 and it is still helpful in today's business environments since it is simple to apply. The model provides an illustration about the whole process of how advertising influence consumer behavior. It's noted that advertising needs to attract attention, maintain interest, create desire, and get action. According to the AIDA model consumer behavior begins from attention to interest, followed by desire and action as the last phase of the model. Figure 1 shows AIDA model.

The AIDA model which displays in Figure 1, consist four different steps to lead consumer to the purchase of goods or services. The four phases of AIDA are as follows:

Attention is the 1st phase refers to the advertisers' efforts to catch the attention of the consumer. If consumer's answer to the first phase is positive, the next step will be formed. Interest, is where a consumer will want to know more about the particular product or service. Afterward, in the next step, Desire may grow which will lead to the purchase of the specific product or service.

Finally, in Action step, the customer moves into actual buying behavior after completing the last 3 phases. According to the AIDA model, some questions were prepared for different phases of the AIDA (Attention- Interest-Desire-Action) separately. Accordingly, participants were asked whether the listed factors below can attract them in order to move to the next step of the model. The question is that the below outlined factors could be desirable to customers' attraction. These factors are listed for each phases of AIDA model.

#### *Attention*

- a) The well designed website
- b) Online advertising messages
- c) Products with new innovation
- d) Convenience and spending shorter time to purchase goods
- e) All time shopping accessibility
- f) The well-known brand name
- g) Words like 'FREE' or 'DISCOUNT' on the seller's website

#### *Interest*

- a) The electronic-catalog through the seller's website
- b) The ease of product's price and quality comparison
- c) The variety of the products
- d) Customer's review availability
- e) The appropriate information, news article, fact about the product
- f) Online guide/support service

- g) Relevant constantly updated website

#### *Desire*

- a) The cheaper /discount price of goods
- b) The special offer like 'BUY ONE GET ONE FREE '
- c) The product grantee and return policy
- d) The picture of the product from different angles
- e) The after – sales services
- f) The shorter delivery period
- g) The high quality packaging
- h) The extra accessories /a small gift
- i) The Experience of free trial product

#### *Action*

- a) The simplicity of buying processing
- b) Personal privacy and security
- c) The 'ADD TO BASKET' sign
- d) Using words like 'BUY NOW 'or CLICK'
- e) Secure payment system like PayPal
- f) The trust of seller's website

#### **Hypothesis**

After a careful review of the literature hypotheses formulated as follows:

H1: Socio-demographic factors have impact on online shopping behavior of British students as well as international students resident in London.

- a) Customers' age has a relationship with online purchase behavior of the consumers.
- b) Customers' gender has a relationship with online purchase behavior of the consumers.
- c) Customers' nationality has a relationship with their online buying behavior.
- d) Customers' household income level of family has a relationship with their online purchase behavior.
- e) Customers' education level has a relationship with online purchase behavior of the consumers

H2: Online purchase behavior of British students differs of the purchase behavior of oversees students.

H3: The viewpoints of British students and international students in terms of advantages and limitations of online purchase behavior are different.

#### **Methodology**

All International master and PhD students resident in an international accommodation in London and equal population of British students studying in a university in London were taken as the population of this study. Since the population of oversees students was limited therefore, the total population was studied. The instrument of data gathering in this study was questionnaire. Questionnaires have been distributed personally face to face among all oversees students on voluntary

basis. Then, 84 respondents were requested to complete the questionnaire.

The online buying behavior items were adopted from AIDA model, (Strong, 1925). In addition, 5-point Likert scale ranging from (1 = definitely disagree, 5 = definitely agree) was used to measure the factors.

Reliability was checked by the help of Cronbach's Alpha. The value of Alpha was 0/94 which confirmed the reliability of the questionnaire. The questions designed according to the four steps of AIDA model including Attention, Interest, Desire and Action. Consequently, data collected from 84 respondents and then responses were fed into the Statistical Package for Social Sciences (SPSS) in order to analysis and evaluation too.

Therefore, the current study consists of a sample group of 84 respondents. In term of gender, 69% of the respondents were male and 31% of the respondents were female. In term of nationality, more than 55% were European, and less than 4% were from North America. 41% of the respondents were between 26 to 30 years old. And 33% of them

were between 26 to 30 years old. All respondents were postgraduate students as 68% were PhD students and 32% studying at master program. In terms of family income level 30% have between 800-1200 (British pound) and 16% have under 800 pound per month. The respondents were also asked about the frequency of shopping online. 62% selected "sometimes" as their answer.

### Result

Regarding hypothesis 1, all five sub-hypothesis are rejected based on their weak relations among the variables involved ( $p > 0.05$ ). With regard to sub-hypothesis 5, marginal findings illustrates that there is a correlation between two first steps of AIDA model (Attract and Interest) with higher level of customer's education at ( $p < 0.05$ ). In other words, demanding online shopping will increase by the higher level of education. The results are displayed in table 1. Table 1 shows Pearson Correlation and education level.

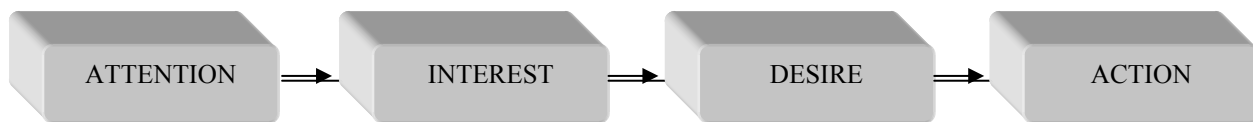


Figure 1: AIDA model

Table 1: Pearson Correlation and education level

		Attract	Interest	Desire	Action	Online shopping behavior
Years of university education	Pearson Correlation	-.246 *	-.251 *	-.050	-.080	-.192
	Sig. (2-tailed)	.025	.022	.651	.470	.081
	N	83	83	83	83	83

\* Correlation is significant at the 0.05 level (2-tailed).

The results also reveal that hypothesis 2 is rejected. Hence, the view point of British students and overseas students regarding the advantages and limitations of purchase behavior are same. Since, t- value is not significant at ( $p > 0.05$ ) for this hypothesis.

For hypothesis 3, as table 2 displays, T-test was used to evaluate. t- Value for 6 advantages out of 8 is not meaningful. But for two of them which are "Time Saving" and "Wider variety of goods to choose" is meaningful at ( $p < 0.05$ ). Table 2 shows T-test, the advantages of online purchase for both groups.

The first factor is exposed as the most advantage of online shopping by international students while British students mentioned the second factor as the

most important advantage when they shop online. Also t-value is not significant for limitations except for two of them. As shown in table 3, they are "Credit card security issue" and "Disappearing of shopping website" where  $p < 0.05$ . Table 3 shows T- test, the limitations of online purchase for both groups.

### The rate of limitations and advantages of Internet shopping

Marginal issues from hypothesis 3 result that British students and International students living in London have different points of view concerning the rate of limitations and advantages through Internet shopping. Freidman test was used in order to rank the limitations and advantages of Internet purchase to clarify which limitation/advantage is most important and which one has less degree of importance.

Based on the results, "Saving time" and "Wider variety of goods to choose" are the most important advantages of Internet buying for International students and British students respectively.

The results of Freidman test also clearly indicate that the most important limitation for overseas students is "Credit card security issue" while British students perceived "lack of touch/feel of goods" as the most significant limitation.

Rating the advantages was also clarified. British students and international student have mentioned the factor of "Saving time" as the first priority of online shopping.

Table 2: T-test, the advantages of online purchase for both groups

	t-test for Equality of Means		
	t	df	Sig. (2-tailed)
1. Convenience at shopping	-1.329	81	.187
2. Cost saving /cheap price	.658	81	.512
<b>3. Time saving</b>	<b>-2.068</b>	<b>81</b>	<b>.042</b>
4. Shopping at any time	-.226	81	.822
<b>5. Wider variety of goods to choose</b>	<b>2.553</b>	<b>81</b>	<b>.013</b>
6. Availability of price comparison	-.726	81	.470
7. No pressure shopping	.488	81	.627
8. No more driving!	1.053	81	.295

Table 3: T- test, the limitations of online purchase for both groups

	t-test for Equality of Means		
	t	df	Sig. (2-tailed)
<b>1. Credit card security issue</b>	<b>-2.278</b>	<b>81</b>	<b>.025</b>
2. Personal privacy issue	-1.880	81	.064
<b>3. Disappearance of shopping website</b>	<b>2.143</b>	<b>81</b>	<b>.035</b>
4. Ordering the wrong item	1.423	81	.158
5. Lack of touch/feel of goods	.276	81	.783
6. Delivery cost	-.037	81	.971
7. Items are delivered not immediately	-.062	81	.950
8. Billing errors	.767	81	.445

### Discussion

According to the results of data analysis, age has not impact on students in their shopping behavior. Scholars have found dissimilar findings regarding age and internet shopping intention (Donthu and Garcia 1999, Rohm and Swaminathan 2004, Stafford et al. 2004). In this study, regarding the four steps of AIDA model, findings show that through the first three stages of Internet shopping including Attract, Interest and Desire, older student having age more than 40 are more attracted to shop online. While, students between 26-30 years approach the last step of AIDA model (Action) earlier than the first mentioned group. In other words, duration of decision making for younger students are shorter compared to older

students. The importance of this is for marketers to distribute appropriate goods for specific age groups.

Looking at gender, in previous researches, researchers and scholars have illustrated that men customers attend to purchases online and spend more money than women (Rodgers and Harris 2003, Slyke et al. 2002, Li et al. 1999, Korgaonkar and Wolin 1999, Stafford et al. 2004, Brown et al. 2003, Alreck and Settle 2002). Further, In Internet shopping, men are looking for known websites to shop, while women visit shopping websites via search engines in order to find their desired products or services (Abrams, 2009). But in this study, gender has not significant impact on Internet shopping.



According to Bellman et al. 1999, Susskind 2004, Liao and Cheung 2001, as well as Bagchi and Mahmood 2004, Education level shows different effects from no effect to a positive effect on online shopping. As illustrated in this study, education level has not influence on shopping online.

Income is a known factor that positively associated to online shopping tendency (Donthu and Garcia 1999, Li et al. 1999, Bagchi and Mahmood 2004, Susskind 2004, Korgaonkar and Wolin 1999). Based on the results, this study does not support the impact of household income level on Internet shopping for both British and International students.

The study showed, the nationality of the students not having any influences on shopping behavior. Because most of the people often thinking the same way as others in the community.

### Conclusion

The results of this research study clearly indicate that there exists a weak association between students' demographic factors including age, gender, household income level, education level and nationality with his/her internet shopping behavior. Additionally, the research conducted here implies that British students and International students have the same shopping behavior.

It is also established through this research that the viewpoints of British students and International students resident in London are different regarding the limitations and advantages of online shopping. "Time Saving" and "Wider variety of goods to choose" are most important advantages of online shopping from International students and British students' perspective respectively. Our results here also offer some information about internet shopping behavior in terms of its limitations. "Credit card security issue" and "Disappearing of shopping website" are mentioned by international students and British students respectively.

While we have mainly focused on consumer demographic factors in an online shopping behavior research, there are other related factors that could be important predictors of consumer behavior. This study shed light on some future research issues. Our suggestions for further studies are as follows:

To assess the link between students' culture and shopping behavior

To examine the relationship between nationality of students and loyalty in online shopping behavior

To assess the association between students' attitude and shopping behavior

To examine the influence of well-known brands on shopping behavior.

To assess the role of students' gender and chosen goods/products in online shopping.

### References

1. Abrams, K.V. "UK men Online", Available on: [http://www.e-marketer.com/Report.aspx?code=emarketer\\_2000571](http://www.e-marketer.com/Report.aspx?code=emarketer_2000571), Accessed on 26 April 2009.
2. Alreck, P. and Settle, R. B., "Gender Effects on Internet, Catalogue and Store Shopping" *Journal of Database Marketing* 2002, Vol. 9, No. 2:150-162.
3. Bagchi, K. and Mahmood, M. A., "a Longitudinal Study of Business Model of On-Line Shopping Behavior Using a Latent Growth Curve Approach." *Proceedings of the Tenth Americas Conference on Information Systems* 2004, New York, NY.
4. Bellman, S., Lohse, G. L. and Johnson, E. J., "Predictors of Online Buying Behavior," *Communications of the ACM* 1999, Vol. 42, No. 12 32-38.
5. Brown, M., Pope, N. and Voges, K., "Buying or Browsing? An Exploration of Shopping Orientations and Online Purchase Intention," *European Journal of Marketing* 2003, Vol. 37, No. 11/12: 1666-1685.
6. Chau, P. Y. K., Cole, M., 45, A. P. M., Montoya-Weiss, M. and O'keefe, R. M., "Cultural Differences in the Online Behavior of Consumers," *Communications of the ACM* 2002, Vol. 45, No. 10: 138-143.
7. Donthu, N. and Garcia, A., "the Internet Shopper," *Journal of Advertising Research* 1999, Vol. 39, No. 3 52-58.
8. Doolin, B., Dillon, S., Thompson, F., and Corner, L. "Perceived risk, the Internet shopping experience and online purchasing behavior: A New Zealand perspective", *Journal of Global Information Management* 2005, Vol. 13, No.2, 66-88.
9. <http://consumers.ofcom.org.uk/2011/12/uk-consumers-are-a-nation-of-online-shoppers/>, accessed on April 2012
10. <http://www.statistics.gov.uk/pdffdir/rs0510.pdf>, cited at: [http://www.ofc.gov.uk/shared\\_ofc/marketstudies/-onlinemarketsdiscussionpaper](http://www.ofc.gov.uk/shared_ofc/marketstudies/-onlinemarketsdiscussionpaper)
11. Hyokjin, K., Fox, R. J., and Zinkhan, G. M. "What products can be successfully promoted and sold via the Internet?" *Journal of Advertising Research* 2002, Vol. 42, No.1, 23-38.
12. Korgaonkar, P. K. and Wolin, L. D., "A Multivariate Analysis of Web Usage," *Journal of Advertising Research* 1999, Vol. 39, No. 2: 53-68.
13. Korgaonkar, P., Silverblatt, R. and Becerra, E., "Hispanics and Patronage Preferences for Shopping From the Internet," *Journal of*

- Computer-Mediated Communication* 2004, Vol. 9, No. 3.
14. Li, H., Kuo, C., and Russell, M. G. "The impact of perceived channel utilities, shopping orientations, and demographics on the consumer's online buying behavior", *Journal of Computer-Mediated Communication* 1999, Vol.5, No. 2: 1-20.
  15. Liao, Z. and Cheung, M. T., "Internet-Based E-Shopping and Consumer Attitudes an Empirical Study," *Information & Management* 2001, Vol. 38, No. 5: 299-306.
  16. O'keefe, R. M., Cole, M., Chau, P. Y. K., Massey, A., Montoya-Weiss, M. and Perry, M., "From the User Interface to the Consumer Interface: Results from a Global Experiment," *International Journal of Human-Computer Studies* 2000, Vol. 53, No. 4: 611-628,.
  17. Park, C. and Jun, J.-K., "A Cross-Cultural Comparison of Internet Buying Behavior," *International Marketing Review* 2003, Vol. 20, No. 5: 534-554.
  18. Park, J., Lee, D. and Ahn, J., "Risk-Focused E-Commerce Adoption Model: a Cross-Country Study," *Journal of Global Information Management* 2004, Vol. 7, 6-30.
  19. Rodgers, S. and Harris, M., "Gender and E-Commerce: An Exploratory Study," *Journal of Advertising Research* 2003, Vol. 43, No. 3: 322-330.
  20. Rohm, A. J. and Swaminathan, V., "A Typology of Online Shoppers Based on Shopping Motivations," *Journal of Business Research* 2004, Vol. 57, No. 7: 748-758.
  21. Slyke, C. V., Comunale, C. L. and Belanger, F., "Gender Differences in Perceptions of Web-Based Shopping," *Communications of the ACM* 2002, Vol. 45, No. 7: 82-86.
  22. Stafford, T. F., Turan, A. and Raisinghani, M. S., "International and Cross-Cultural Influences on Online Shopping Behavior," *Journal of Global Information Management* 2004, Vol., 7, No. 2: 70-87.
  23. Susskind, A., "Electronic Commerce and World Wide Web Apprehensiveness: An Examination of Consumers' Perceptions of the World Wide Web," *Journal of Computer-Mediated Communication* 2004, Vol. 9, No. 3.
  24. Teo, S. H. T. "Demographic and motivation variables associated with Internet usage activities," *Internet Research* 2001, Vol. 11, No.2, 125-137.

**Crime in Children and adolescents**Ommolbanin Falahatibejarpas<sup>1\*</sup>, Seyedahmad Beheshti<sup>2</sup><sup>1</sup> Khomein Branch, Islamic Azad University, Khomein, Iran. [toloue90@gmx.com](mailto:toloue90@gmx.com)<sup>2</sup> Khomein Branch, Islamic Azad University, Khomein, Iran

**Abstract:** This research has been designed and executed with the objective of studying the role of parents' training to their incompatible adolescents in order to create compatibility in counties of Tehran Province. At first teachers and officials of six girls, guidance schools in Tehran counties were requested to introduce their incompatible students according to the factors of Behavioral Assessment Questionnaire of Kanrez. In the next stage, the parents of incompatible students are invited to answer the form of parents' opinion about their adolescents' behavior at home related to behavioral assessment questionnaire of Kanrez. In case the opinions of both groups (parents and teachers) are somehow the same for diagnosis of adolescents' incompatibility, the incompatible students are listed in the final list of sample group. Therefore, 24 persons were selected as samples and they were divided randomly in two test and control groups. But six guidance schools were selected randomly among the girls' guidance schools in Tehran counties. The methodology of applying the extant paper is pretest and posttest semi-experimental researches without random selection including comparing the behavioral performance of test group with control group. So that at first, both test and control groups are tested with respect to the behavioral assessment questionnaire of Kanrez, parents form as pretest and then for 2 months with the presence of parents of test group the sessions of parents training were held and in the final stage, test group and control group were analyzed via behavioral assessment questionnaire, parents form (which has been standardized among the adolescents of girls, guidance schools of Tehran counties) and has been applied as their posttest. Analyzing the results via t test indicated that statistically no significant difference within the range of 0.01 has been observed between the averages of scores for pretest and posttest of each one of the test and control groups and this means that family's training regarding the parents of criminal incompatible adolescents is effective on their compatibility. Since at present the number of adolescents and youths under 24 years old in our country forms about 1/3 of the whole population and consequently, Iran is considered as the young countries in the world, identifying the behavioral problems of adolescents and youths and creating the best and most appropriate interaction methods for promoting the psychic health of this age group has been very important.

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**Keywords:** Adolescents, Compatibility, Crime, Family training, Incompatibility, Parents

**1. Introduction**

The beginning of human life- the same germinal life - is a period that the germ is dependent purely on mother and it gradually goes towards independency so that in the next periods the dependency is faded and instead the independency becomes outstanding and obvious. Among the human growth periods the adolescence is very important because it is the last growth stage of children during which the parents and instructors may help them actively in order to find their way in life; it is the last stage that the parents can be pattern for them; proceeded with family activities and provide suitable opportunities and have direct contact with training process. At the end of this period, most of adolescents go to university, seek for job, marry and briefly enter into their independence world (BnJ. (2003)). Adolescence has been deemed either as the viewpoint of adolescents or the parents since years ago this period has been more difficult than childhood and it has been considered as a conflict

stage and sometimes life difficult period. Why? The first and the clearest answer is that adolescence particularly the first years is the period of physical, sexual, mental and cognitive as well as social requirements changes. It is unfair that exactly when the teenager passes the physical adolescence with an unprecedented speed, the community asks him to be independent. So the adolescents not only have to deal with growth they also changes should attempt to gain an identity for themselves and find a personal answer for this question that "who I am"? The crime is the differences between sexual compatibility and other incompatible behaviors. The adolescents who can act diverse social and individual roles are socially and emotionally more prepared for future life and marriage. (Bernstein .G.A..P.H.Svingen and B.D. Garfinkel(2000)). The adolescence is a period of experiencing extensive changes in cognitive, neural-biological and social contexts. One of the subjects which must be highly considered in the adolescence is identification of their compatibility characteristics and it must be noted that any leakage in this context

may lead to harmful consequences such as crime. Perception of compatibility styles may be effective on clinical therapy (particularly counseling), educational planning, administrating the course classes and identification of growth period of the adolescents (Frydenberg & Lewis, 1993). Compatibility is a general concept which is referred to any strategy that a person uses for administrating the stressful situations in life including real and unreal threats. (Sadok & Sadok, 2003). The compatibility has two main actions: when a problem is confronted it is called as "problem-oriented compatibility" and when it is attempted for pacifying the emotional states it is called as "emotion-oriented compatibility". The efficiency of compatibility becomes significant in the context of structure or existing conditions. In confronting the mental pressures due to occupational or economical problems, the methods which make the person far from the problem are more effective but about the problems resulted from interpersonal relationship, the methods that involve the person in problems more, have more efficiency (Folkman & Lazarus, 1985). The difference between compatibility resources of people is dependent on the history of individual life, social and cognitive growth, prestress experience, motivation system, beliefs and the structure and sensitivity of the receptors (Ritchie, Matrin H. (2005)). Cognitive-behavioral method has extensive application for helping the people for better administrating the stress replying. Via this method, a pattern based on the concepts of initial evaluation (evaluation of an event meaning) and secondary evaluation (what can do about that event) is applied (Dimsdale, Keefe & Stein, 2000). The effectiveness of these methods for improving the compatibility and remission of the people affected by chronic diseases has been proved (Parker et al, 1995; Milne, Joachim & Niedhardt, 1986)

### 1.1. Explanation of subject

Doubtless the requirement for any cultural deep and integrated mutation in each society is basic changes and evolution in its educational system and essential mutation in the educational and training programs of the schools is subject to the compatibility of value system applicable for the families and schools and mental readiness and tendency of the parents for accompanying with the planners and instructors of the schools. Most people reprove the parents due to having uncomfortable, vicious, restless and unsuccessful treatments, and the problems they may create. Even the experts of education and training after precise perusing and studying the statistics related to the adolescents having emotional-psychic problems or probably victims of crime, reprove and rebuke the parents and

often deem them as guilty. Here this essential question is raised that who helps the parents and what effort and policies are applied for assistance and education towards presenting the effective methods for training their children? Therefore and considering the importance of answering these important and determining questions the following question is raised for the researcher: if the family training is effective on creation of compatibility for incompatible and criminal adolescents? Is the family training effective on psychic health of the family?

### 2.1. The importance and necessity of the research

The adolescents and youths form a great population all over the world. The estimation provided by Ionesco statistics in this context indicate that within 1960-2000, the age group of 15-24 has been exceeded from 415 million persons to 1280000000 persons. Furthermore, the most number out of this amount are living in third world and our country Iran forms about 1/3 of this population, this active and gross population may not be ignored (Loeber, R. (1990)). The adolescents and youths' problems are different in various societies and cultures. The problems of rural adolescents is not similar to adolescents problems, even the adolescents and youths living in a big city follow the different micro-cultures and have various social bases but have not the similar problems. Principally what is considered as a problem in a society may be not a problem in another society. The problems of this period are sometimes connected to each other like chain rings and each one led to another one. Increasing the problems is a danger that threatens the adolescent and youth and makes problems for them. If these problems are not removed, other problems may be created and consequently led to individual and social deviation, crime and other undesirable outcomes (Taylor, J., McGue, M., & Lacono, W. (2000)). The family is the first and most important base for growing the adolescents' emotions. Social, economical and cultural status, the parents' educational level, customs, family members, wishes and ideals of the parents are considered as the effective factors on the growth of adolescents' emotions. The adolescent should release the thought, dependencies and relations of childhood and achieve the new skills; the successful transfer from childhood to adolescence requires changing the concept of self and this is not possible without the assistance of the acquaintances and particularly well-informed parents. Here the adolescent needs the guidance of well-informed people for conformity with the environment and identification and evaluation of the acquaintances. The family due to close contact with the adolescent may lead him towards the ingenuity and sincerity or hatred and hate. If the family

understands its role in the adolescents training and learn and apply the appropriate method, many of behavioral and emotional disorders will not be created (Frydenberg, E.(1997).

### 3.1. The research objective

Reviewing the role of family training in creation of compatibility for incompatible (criminal) adolescents studying in girls' guidance schools of Tehran counties has done.

### 4.1. Research hypothesis

This research includes one objective and main hypothesis:

The family's training is effective on creating the compatibility for incompatible girl adolescents studying the guidance school course.

### 5.1. The research literature and review

In this section, at first the principle concepts and theoretical fundamentals related to the research subject are raised and then the studies and researches applied in Iran and foreign countries in this regard are explained briefly. The family training is a type of group education which includes an expanded educational plan and helps them to promote their knowledge and capabilities in the context of training the child and adolescent desirably. The family training is preventive extensively and prevents from creation of social and emotional incompatibilities and helps the progress and improvement of child and adolescent's psychic health. In family training like individual training, it is attempted to introduce the principles and concepts of child and adolescent growth and conducting the child and adolescent in different states and identify the child and adolescent's problems in different ages of life and learn the conduct modes. Adolescence is referred to as a transient period from childhood to adulthood. This period has no specified age range but is beginning from 12 and is continued until the last years of second decade of the life in which the physical growth is completed somehow. In this period the adolescent achieves the sexual maturity, constitutes his individual identity independent from his familial identity and is confronted with his subsistence affairs (Connors.C.K.E.(2005)). The adolescence is a period between childhood and adulthood that its range and duration is not specified and varies depending on different people and societies. Its commencement is concurrent with sexual maturity but commonly may state that the adolescence lasts from 12 years old until almost 18 to 21 years old (Parker,J.C. et.al.(1995)). Connors.C.K.E (2005) has studied the relationship between child training by parents and their self-esteem. The results of this research indicated that a high positive relationship exists between logical power to the opinionated and left-freely, independent

and targeted children and they have more self-confidence and responsibility. Ritchie,Matrin H (2005) have applied a research on 213 school consultants regarding the determined range and the nature of development of consultants in the context of parents' skill and training and counseling. According to this research it was concluded that still the communication with the parents is weak and the consultants don't spend enough time for communicating with the parents (counseling services).

Taylor,J.,McGue,M.,&Lacono,W.(2000) as respect to the parents' training have applied a study on 31 young spouses and stated the results are useful. Tennies,Robert H.A.(2002) was divided into three categories upon evaluation of communication program and contact of the parents in the Bokaraton Armenian school whose children were troubled. The communication of each one of the parents group with school led to the improvement of children and also higher educational progress. Cok et al (1991) have analyzed the emotional style of parents and family system. In this research the social communication model for clearing the dynamisms related to the parents' emotional style has been used as the guideline for parent conduct with the child. The results gained from 70 families who were seeking for clinical services for an adolescent member indicated that firstly motivate the emotional style of their parents so that take them at risk and secondly this negative emotion stimulation of the parents helps to expressing the negative emotion of the adolescents to their parents. The pattern that can show the contexts of crime better of all is a cumulative pattern (Loeber, 1990), Taylor, Mc. Gue and Racono (2000) have estimated that the role of uncommon environment in appearing low and medium crime is 56%, the role of genetics 1% and the role of common life traits 26%. Satter .I.M.(2004) through comparing the compatibility styles of prisoners in public courts of Varamin with the control group including employees of this prison concluded that the prisoners in comparison with the employees are not different as respect to the application of conforming strategies (rational and separate) but the rate of utilization of nonconforming strategies (emotional and avoidant) is more. The jailed women applied nonconforming strategies significantly more in proportion to the jailed men.

Hill, H.M. et.al (1996) in a research, has taught the cognitive-behavioral approach to the parents of the adolescents who were referred by the supportive organizations via group method. In this method, patterning, feedback, social reinforcement, giving homework methods were applied. In these sessions, the wrath controlling and replacing

inappropriate behaviors instead of them were discussed. The results indicated that the taught parents after training have showed fewer wraths in conducting the adolescents, after training. Rosenberg et al (2003) in their applied study have reviewed the responsibility of the students and communication between parents-children or their educational performance in the guidance school course. A group consist of 120 families with the first child whose age is within 11 to 16 have participated in this research. Evaluation of adolescents' responsibility including the rate of self-reliance, professional tendency, strengthens against the pressure of the same age adolescents and participation in familial responsibility. Generally, the sectional studies indicated that the adolescents' being responsible is related with the parents' action which has been described as power and educational success. But the researches indicated that the responsibility is a correlation but not a pre-occurrence, educational success increases the parents' power, therefore it is not still clear that an adolescent whose parents are rather easy dealing, more acceptor and had less control psychologically, had better performance in school than the other classmates (Rosenberg et al, 2003).

## **2. Material and Methods**

### **1.2. Statistical society, sample and sampling method:**

The sampling group in this research is consisted of all girl students studying in one of the grades of the guidance school of Tehran Counties in the academic year 2009-10. The samples were 24 persons of incompatible students in the guidance school course which were selected among the sampling group members via multistage cluster sampling method. According to this method, among the counties of Tehran the Education Departments of two Varamin and Boumehen counties were selected randomly and a list of girl guidance schools was provided, then three schools of each district (altogether 6 schools) were selected via simple random sampling method and in the last stage 24 persons were selected as sample among the selected schools and considering the respective factor and were divided randomly in two test and control groups. The procedure was in a way that after choosing three schools of each county via simple random method, we approached these schools and discussed with the relative officials regarding the research and the characteristics of the adolescents who should be analyzed as the examinee. In the next stage, the teachers of the selected students were asked to consider the factors of behavioral assessment questionnaire of Kanrez, answer the parents form and

state their personal answer and pursuant to which the parents of students of three schools were invited and requested to answer the behavioral assessment questionnaire of Kanrez, parents form which had been standardized in this age range by the researcher in Tehran counties, guidance school course. In case the students were diagnosed as incompatible adolescents, by parents and teacher, they were considered as samples. Therefore 24 incompatible students were selected as samples out of 6 schools of two Tehran counties, among this range 12 persons were selected as test group and 25 persons as control group for participation in the research.

### **2.2. Research methodology**

The methodology applied for this research is semi-experimental method, pretest and posttest plan with control group. This research has been applied with the objective of studying the effect of family training for creating the compatibility for incompatible adolescents. The general purpose of the research is comparing the effect of family training on the test group (incompatible adolescents who have been identified as the incompatible adolescent by the school officials and teachers and their parents have been trained sufficiently in this context) with control group (incompatible adolescents who have been identified as incompatible adolescent by the school officials and teachers but their parents have not been trained sufficiently in this context). In this paper, family training is independent variable and adolescents' incompatibility is dependent variable. Family training means offering psychological information regarding adolescence, familiarizing the parents with the educational, emotional and social problems as well as the mode of emotional, social, religious and also sexual training and the appropriate communication with them and the educational environment (school). Moreover familiarizing with the counseling services, during the group training sessions, the parents are taught how to promote their student and his capabilities in the context of education and training desirably. In this paper, the family training sessions were held for 12 sessions each one 75 min. At first, about 20 to 30 min, the concepts and principles of adolescent growth and the procedure of conducting with the adolescent regarding the incompatibilities and problems of this period have been taught via speech and workshop methods by the family training instructor and then for 20 to 30 min the techniques and behavior therapy have been taught to the parents. During the training course, after every two or three sessions, one session has been allocated to the question and answer session and reviewing the training in the previous sessions. In these sessions, the family training instructors were utilized in Tehran and its counties. Also, in the extant

research according to the presented factors available in the behavioral assessment questionnaire of Kanrez, parents form which has been stabilized by the writer within the age range of 12 to 15 years old of students studying in the guidance school of Tehran counties and based on the scores they have gained for this question, the vicious adolescents have been identified as incompatible adolescents.

### 3.2. Measurement tools

In order to gather the data for this research, the behavioral assessment questionnaire of Kanrez, parents form has been used for identification of incompatible students. For executing the mentioned questionnaire, at first the teachers of the students were requested to state their opinions based on the introduced factors in the questionnaire accurately and according to their observations. Then the parents (mainly mothers) of the students who have been identified as incompatible adolescents were invited separately to attend in an assembly at school.

### 4.2. Assessment questionnaire of Kanrez, parents form

The questionnaire applied in this research has been presented by Kanrez in 1969 and is consisted of 48 articles and includes an extensive part of behavioral problems common among the children suffer from behavioral disorders. This list of behavioral symptoms is answered by the parents according to their observations and then the information about the children and adolescent's behaviors. In this questionnaire, four degrees of behavioral problem's strength and weakness have been considered. The parents (particularly the mothers) are asked, in case of non-existing a behavioral problem in the adolescent, mark the first option. If a behavioral problem exists but it is less intense, mark the second option. If behavioral problem exists and its intense is average, mark the third option and finally if the problem severity is high, mark the fourth option. While scoring this questionnaire, if the first option (nothing) has been marked, no point is applied. If the second option (a little) has been marked, one point is applied and if the third option has been marked as (average) two points and finally if the fourth option has been marked, three points are applied. The total gained points in each questionnaire forms the total score of behavioral symptoms list. The scores gained from each questionnaire and in the basic examinee is used for reviewing the differences existing between the examinees in a group and comparing these groups in the pretest and posttest. The studies applied on the parents' grading scale of Kanrez indicate that it has sufficient validity and reliability (Garmez, N. 1993). Furthermore, the provided factorial analysis supports the first five provided factors by the scale. It has been

proved that Kanrez's grading scale has an appropriate internal stability, retest reliability and judge reliability. Also, statistically a significant correlation exists between teacher and mother-father (Garmez, N. 1993). This tool has been used in many cultures extensively (Osofsky, J.D. 1995). The behavioral problems which have been sampled by grading scales of Kanrez have been found with different frequency degrees in all studies (Jaffe, P.G., Wolfe, D., and Wilson, S. 1984).

## 3. Results

### 1.3. Data analysis

In this stage the tables of raw scores, the form of reliability index rate have been calculated via Cronbach's alpha coefficient and formula of sum totals. Through applied factorial analysis the factorial structure gained from the scales has been studied and then the validity index of the test has been analyzed. Statistical analysis of this research and standardization of measurement tools have been applied aiding SPSS software. In the mean statistical method, the gained standard deviation is calculated via descriptive statistical methods and many diagrams have been exhibited. Also, by inferential statistics and t test for the independent and dependent mean values the significance of difference between mean values of difference of pretest, posttest scores of both test and control groups and pretest and posttest of each one of test and control groups were examined.

**Table 1:** it has shown t test of independent groups on the counseling effectiveness with the cognitive-behavioral approach on the compatibility of incompatible adolescents

	Difference between the mean values	Standard deviation	Factor error	Ratio	Degree of freedom	Alpha rate
Test	1.02	1.8	0.51			
Control	7.12	7.2	2.1	8.1	22	1%

As it is observed the group counseling with the cognitive-behavioral approach is effective on a family that has an incompatible adolescent. The comparison of average scores indicates that tendency to the incompatibility in the test group has been reduced significantly. For determining the stability, the effect of group counseling with the cognitive-behavioral approach on the compatibility of incompatible adolescents during the time, correlated t test has been used for test group, in posttest and following stages.

**Table2:** it has shown Correlated t test for comparing the scores average of test group compatibility in posttest and pursuit stages

Stage	Mean value	Standard deviation	Standard error	T test	Degree of freedom	Alpha rate
Test group	60.5	5.3	105			
Pursuit group	60.17	5.02	1.4	0.93	11	0.36

As it is observed no significant difference exists between compatibility of incompatible adolescents of test group in posttest and pursuit stages and it certifies this fact that the effect of group counseling on the compatibility of incompatible adolescent has a relatively appropriate stability over time. Considering the results of this study, undoubtedly the family is the most important and major education and training center for everyone and it is considered as one of the educational and training institutions of the society. Therefore, explaining any thought and word regarding the training of individuals particularly the children, adolescents and youths is not correct without considering the vital role of the family. Therefore, the parents training, holding training sessions for family training, using family therapy method and studying the status and success of adolescent or youth in the family are ever introduced as preventive strategy. All these efforts are rooted in this thought that an effective, dynamic and healthy system is a system that should benefit from the empathy, sympathy, cooperation and coordination of health, aware and lively families. Thus all these efforts are based on this principle that the "capability" of mothers, fathers, teachers, training instructors and planners for education and training of children and adolescents- these real capitals of the society- rely on their awareness and knowledge on principles, techniques and art of education, psychology and other relevant science. It seems that the first and the most important step in the education process is familiarizing the parents and teachers with the principles and methods of making right human relationship and investment on family training. So in this course, it should be confirmed that children are primarily the training product of family and the roots of future race's personality are formed in the family.

#### 4. Discussions

In this research during 12 sessions of family training, the following concepts and instructions will reduce the incompatibility of adolescents:

- 1- Familiarizing the parents with the content and process of sessions;
- 2- Informing the parents of the history of studies and the opinions of experts on adolescence;

- 3- Introducing the application of behavior improvement principles;
- 4- Familiarizing the parents with the child training methods;
- 5- Informing the parents of the necessity of discipline in the socialization process of the adolescent;
- 6- Informing the parents of deficient relationships between parents and children and the effect on the adolescent's personality;
- 7- Introducing the growth traits in the adolescence ( 12 to 15 years old);
- 8- Training the stability to parents so that the adolescent understands how to react against special behavior;
- 9- Training the parents the principles and methods of communicating with children;
- 10- Familiarizing the parents with methods of awarding the desirable behaviors of children;
- 11- Familiarizing the parents with punishment methods for children;
- 12- Familiarizing the parents with self concept and identity growth in the adolescent, psychological problems of the adolescence and reduce of inappropriate behaviors.

In the last session, at first a summary of all previous sessions was presented and the questions of participated members were answered.

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#### References

1. Bernstein .G.A..P.H.Svingen and B.D. Garfinkel (2000). School patterns of Family Functioning .Journal of American academy of child and adolescents psychiatry.29.24.30.
2. BnJ.(2003). Parental Authority Questionnaire .Journal of Personality assessment 57.110.119.
3. Conners.C.K.E.(2005). A Teacher Rating Scale for Use in Prug Studies with Children.Amer J.Psychaiatry.126 December 1969.pp.884,888.
4. Cook.W.L..D.A.Kenny and M.J. Goldestein (1991). Parental Affective Style Risk and the Family System: ASchool Relation Model Analysis.Journal of Abnormal psychology .100.492-501.



5. Folkman, S., & Lazarus, R. (1985). If it changes, it must be a process: Study of emotion and coping during three stages of a college examination. *Journal of Personality and Social Psychology*, 48, 150-170.
6. Frydenberg, E. (1997). *Adolescent coping: theoretical and research perspectives*. London: Routledge.
7. Frydenberg, E., & Lewis, R. (1996). A replication study of the structure of the Adolescent Coping Scale: Multiple forms and applications of a self-report inventory in a counseling and research context. *European Journal of Psychological Assessment*, 1, 224-235.
8. Frydenberg, E., E., & Lewis, R. (1993). *Adolescent Coping, Scale: Administrator's manual*. Hawthorn, Australia: The Australian Council for Educational Research.
9. Frydenberg, E., E., & LeWis, R. (1991). Adolescent coping: The different ways in which boys and girls cope. *Journal of Adolescence*, 14, 113-119.
10. Garmezy, N. (1993). Children in poverty: Resilience despite risk. *Psychiatry* (February 1993), 56:127-35.
11. Hebeck, B., Neill, J. T. (1999). Stability and change of adolescent coping style and change of adolescent coping style and mental health. Paper Presented to the Australian Human Development Association Conference, Sydney, Australia.
12. Hill, H. M., Levermore, M., Twaite, J., and Jones, L. (1996). Exposure to community violence and social support as predictors of anxiety and social and emotional behavior among African-American children. *Journal of Child and Family Studies* (1996) 5:399-414.
13. Jaffe, P. G., Wolfe, D., and Wilson, S. (1984). *Children of battered women*. Newbury Park, CA: Sage Publications, 1990; Carlson, B. E. Children's observations of interparental violence. In *Battered women and their families*. A. R. Roberts, ed. New York: Springer Publishing, 1984, pp. 147-67.
14. Loeber, R. (1990). Development and risk factors of juvenile antisocial behavior and delinquency. *Clinical Psychology Review*, 10, 1-41.
15. Noller, Patricia and Robert Taylor (2004). Parent Education and Family Relations. *Journal Article*, Vol. 38, n2, p196-200 Apr.
16. Osofsky, J. D. (1995). Children who witness domestic violence: The invisible victims. *Social Policy*.
17. Parker, J. C., Smart, K. L., Buckelew, S. P., Stucky-Ropp, R. C., Hewett, J. E., & Johnson, J. C. (1995). Effects of stress management on clinical outcomes in rheumatoid arthritis. *Seminars in Arthritis Rheum-atis*, 38, 1807-1818.
18. Ritchie, Matrin H. (2005). Parent Education and Consultation Activities of School Counselors. *Journal article*. Vol. 41, n3, p165-170 Apr.
19. Rosenberg, Leon A. and Sushma Jani (2003). Cross-Cultural Studies with the Conners Rating Scales. *Journal of Clinical Psychology* November, 2003. Vol. 51, No. 6.
20. Sadock, B. J., Sadok, V. A. (2003). *Synopsis of psychiatry*. Philadelphia: Lippincott Williams & Wilkins.
21. Satter, I. M. (2004). *Assesment of Adaptive Behavior problem*. Sandiago, publisher. Inc
22. Satter, I. M. (2004). *Assesment of Adaptive Behavior problem*. Sandiago, publisher. Inc.
23. Taylor, J., McGue, M., & Lacono, W. (2000). Sex difference assortative mating, and cultural transmission effects on adolescent delinquency: Twin family study. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*, 41, 433-440.
24. Tennes, Robert H. A. (2002). *Paternal Involvement Program Including Communication to parent Integrated with a parent Integrated with a parent-Education program and its Effects on Academic Achievement*. Vol. 1, N. 1, p. 7-13 Fall.

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## The Possible protective effects of *Physalis peruviana* on carbon tetrachloride-induced nephrotoxicity in male albino rats

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**Abstract:** *Physalis peruviana* (physalis) has long held a place in folk medicine in the tropical countries where it grows. Here, the aim of this study was to evaluate the potential nephroprotective impact of physalis extract against carbon tetrachloride (CCl<sub>4</sub>)-induced kidney injury and to explore the possible mechanisms by which this plant exerts its beneficial effects. Rats were randomly divided into equal four groups, eight rats each. **I. Control Group:** served as Control group. **II. Physalis group:** rats were treated with Physalis extract in drinking water at a dose of 150 mg/kg b.wt. **III. CCl<sub>4</sub> group:** rats were treated with CCl<sub>4</sub> at a dose of 2 mL/Kg b.wt. and **IV. CCl<sub>4</sub> and physalis group:** rats were treated with Physalis extract in drinking water and CCl<sub>4</sub> at the previous doses for 12 weeks. At the end of the experiment, blood samples were collected and used for determination of kidney function, namely; uric acid, urea and creatinine, while the kidney tissues were subjected to hematoxylin and eosin and Bcl-2 immunostaining examination. Evaluation of malondialdehyde (MDA), nitric oxide (NO), glutathione (GSH) were conducted and the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) were carried out. Animals treated with CCl<sub>4</sub> exhibited significant elevations in kidney function, MDA, NO and exhibited significant decrease in the activities of SOD, CAT, GPx, GST and GSH contents. The combination (both physalis and CCl<sub>4</sub>) group has preserved the kidney histology, kidney function near to control, exhibited a significant induction in the activities of CAT, SOD and GST, increased the kidney content of GSH and Bcl-2 and conversely showed significant decrease in kidney MDA and NO levels compared to CCl<sub>4</sub>-treated rats. Physalis confers an appealing nephroprotective effect which might be explained partially via diminishing the generation of MDA and NO, induction of antioxidant systems and Bcl-2.

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**Keywords:** Carbon tetrachloride; *Physalis peruviana*; Nephrotoxicity; Rats

### 1. Introduction

Oxidative stress results from the imbalance of reactive oxygen species (ROS) and defense mechanisms which results in cell damage. Reactive oxygen species (ROS), including superoxide radicals (O<sub>2</sub><sup>•-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and hydroxyl radicals (OH<sup>•</sup>) are generated as byproducts of normal metabolism (Rice-Evans and Miller, 1996). Cumulative oxidative damage leads to numerous diseases and disorders (Halliwell, 1991). The enhanced production of free radicals and oxidative stress can also be induced by a variety of factors such as radiation or exposure to heavy metals and xenobiotics e.g. carbon tetrachloride (Kim *et al.*, 1990).

Carbon tetrachloride (CCl<sub>4</sub>) is an industrial solvent with a strong nephrotoxin, extensively used to induce oxidative stress in laboratory animals. CCl<sub>4</sub> toxicity results from its bioactivation to trichloromethyl free radical by cytochrome P450 isozymes (Raucy *et al.*, 1993). The trichloromethyl radical reacts with oxygen to form the highly toxic reactive trichloromethyl peroxy radical, a reactive oxygen species (ROS). Free radical-induced lipid peroxidation

believed to be one of the major causes of cell membrane damage, depletion of antioxidant status and DNA injuries in kidneys of rat leading to a number of pathological situations (Khan *et al.*, 2010). Reports from Ogeturk *et al.*(2005b) suggested that acute and chronic renal injuries occur due to the exposure to carbon tetrachloride. Kidney tissue has great affinity for CCl<sub>4</sub> because of the predominant presence of the cytochrome p450 in the cortex. Previous reports suggest that CCl<sub>4</sub> generates free radicals with the implication of pathological environment by damaging the integrity of cell membranes, elevating thiobarbituric acid reactive substances (TBARS) level with subsequent necrosis and affecting physical parameters of kidney such as urinary and serum profile (Sahreem *et al.*, 2011). Renal sources for ROS are activated macrophages, vascular cells, and various glomerular cells. ROS may affect cells of the host organism, especially at sites of inflammation in addition to playing a role in the defense system against other agents. This effect plays a role in a variety of renal diseases such as glomerulonephritis and tubulointerstitial nephritis which can contribute to

proteinuria and other conditions (Ichikawa *et al.*, 1994). The presence of inflammation is well-documented factor influencing the development of oxidative stress in dialysis patients (Samouilidou *et al.*, 2003).

It has been noticed that many of plants which are rich in phenolic compounds, are widely used as antioxidant and antimutagenic (Sivalokanathan *et al.*, 2006). High consumption of fruits and vegetables is associated with low risk for several degenerative diseases, such as coronary artery disease, stroke, rheumatoid arthritis, diabetes and cancer, which is attributed to the antioxidant vitamins and other phytochemicals (Prior, 2003).

*Physalis peruviana* Linn. (Solanaceae) is an erect branching densely villous perennials, which are grown for their fruits and for decoration. They grow wild in Europe, America and Asia. It has been used as a folk medicine with antiinflammatory, antitussive, antipyretic, diuretic, antidotal and antitumor effects in Taiwan (Lee *et al.*, 2008). Various chemical compounds like 28-hydroxywithanolide, withanolides, phygrine, kaempferol, and quercetin di- and triglycosides are reported to be present in *Physalis peruviana* (Arun and Asha, 2007). Some of these components were found to have antitumor, cytotoxic inhibition of the ubiquitin-proteasome pathway (Ausseil *et al.*, 2007), immunomodulatory (Soares *et al.*, 2006), antimycobacterial (Pietro *et al.*, 2000), antiinflammatory and antiallergic activities (Lee *et al.*, 2008).

*Physalis peruviana* contains biologically active components e.g. physalins, withanolides, phytosterols and polyunsaturated fatty acids e.g. linoleic acid and oleic acid. Among its major components are high amounts of vitamins A, B and C as well as the presence of essential minerals, magnesium, calcium, potassium, sodium and phosphorus which are classified as macronutrients, while the Iron and Zinc are considered as micronutrients (Szefer & Nriagu, 2007).

The fatty acids composition and high amounts of polyunsaturated fatty acids found in oils extracted from *physalis peruviana* L. make this fruit ideal for nutrition (Ramadan and Morsel, 2003).

The bioactive phytosterols would give them properties such as antioxidant and hypocholesterolemic effects. Furthermore, the antioxidant activity is due to the high levels of polyphenols and high levels of vitamins A and C. Finally, the presence of exclusive *Physalis*-gender Physalins and withanolides specific from the Solanaceae family would give the fruit of *physalis peruviana* L. anti-inflammatory, antimicrobial and anticancer properties.

The withanolides are steroidal lactones mainly produced by Solanaceous plants. Its components have antimicrobial properties, antitumor, anti-inflammatory, hepatoprotective or immunomodulatory and

antiparasitic activity (Ahmad *et al.*, 1999; Lan *et al.*, 2009).

The specific active constituents of *physalis peruviana* L. are Physalins A, B, D, F and glycosides, which show anticancer activity (Wu *et al.*, 2004). Physalins are immunosuppressive substances which are widely used to inhibit unwanted immune responses in autoimmune diseases, allergies and organ transplants.

The fruit of *physalis peruviana* L. is highly nutritious, having high levels of vitamins A, B and C. The main active components of vitamin A in fruits are  $\alpha$ -carotene,  $\beta$ -carotene and  $\beta$  cryptoxanthin (Fischer *et al.*, 2000).

Phytosterols are of great interest because of its antioxidant capacity and impact on both total cholesterol and LDL cholesterol (Ramadan and Morsel, 2003; Valenzuela & Ronco, 2004).

The present study aims to investigate the possible protective effect of *Physalis peruviana* extract against carbon tetrachloride-induced nephrotoxicity and apoptosis.

## 2. Materials and Methods

### 2.1. Animals

Adult male Wistar albino rats weighing 120–150g (7-9 weeks old) were obtained from The Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). Animals were kept in wire bottomed cages in a room under standard condition of illumination with a 12-hours light-dark cycle, 55±5% relative humidity and at 25±2°C room temperature. They were provided with balanced standard pellet (VACSERA) as a diet and tap water *ad libitum*. The animals were kept in a clean and low stress environment with an enclosed door. The experiments were approved by the state authorities and followed Egyptian rules on animal protection.

### 2.2. *Physalis peruviana* extract preparation

Fresh fruits of *physalis* were purchased from local market in Cairo. The samples were identified in Botany Department, Faculty of Science, Helwan University. The fruits were cleaned, dried and minced, the juice and residue were used for the preparation of crude methanolic extract by percolation at room temperature with 70% methanol alcohol and kept in refrigerator for 24 hours. Extract of *physalis* was concentrated under reduced pressure (bath temperature 50 °C) and dried in a vacuum evaporator. The residue was dissolved in distilled water, filtered and used in experiments.

### 2.3. Measurement of flavonoids, total polyphenols and *in vitro* free radical scavenging assays

For the assessment of flavonoids, colorimetric method introduced by Dewanto *et al.* (2002) was adapted. To determine the amount of flavonoids by the above mentioned method, 1.50 ml of the deionized water was added to 0.25 ml of the sample and then 90  $\mu$ l of 5% Sodium nitrite (NaNO<sub>2</sub>). Six min later, after

addition of 180 µl of 10% AlCl<sub>3</sub>, mixture was allowed to stand for another 5 min before mixing 0.6 ml of 1M NaOH. By adding deionized water and mixing well, final volume was made up to 3 ml. Using blank, absorbance was measured at 510 nm. Calibration curve was prepared using quercetin acid as standard for total flavonoids which was measured as mg quercetin equivalents (QE) per milliliter of the sample (µg/ml).

The total polyphenolic contents (TOC) were measured using Folin-Ciocalteu reagent by the method of Kim *et al.* (2003) based on the oxidation of polyphenols to a blue colored complex with an absorbance maximum of 700 nm. Calibration curve was prepared using gallic acid as standard for TPC which was measured as mg gallic acid equivalents (GAE) per milliliter of the sample (µg/ml).

The free radical scavenging capacity was evaluated by 2, 2-Diphenyl -1- picrylhydrazyl (DPPH) assay described by (Burits and Bucar, 2000). In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species, the absorption decreases. Briefly, 1ml of 0.25 mM solution of DPPH in methanol was added to 50, 100, 150 and 200 µl of sample in 950, 900, 850 and 800 µl methanol, respectively. After 20 min, the absorbance was measured at 517 nm. Ascorbic acid was used as a reference standard. The percentage DPPH decolorisation of the sample was calculated by the equation: % DPPH scavenging =  $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$ , where A is the absorbance.

The total antioxidant potential was measured by the ability of the sample to scavenge thiobarbituric acid-reactive substances (TBARS) (Tripathi and Sharma, 1998). Briefly, 50, 100, 150 and 200 µl of the different samples were added to the 10% liver homogenate. Lipid peroxidation was initiated by addition of 100 µl of 15 mM FeSO<sub>4</sub> solution to 3 ml of liver homogenate (final concentration was 0.5 mM). After 30 min, 100 µl of this reaction mixture was taken in a tube containing 1.5 ml of 0.67% thiobarbituric acid (TBA) in 50% acetic acid. Samples were incubated at 37°C for 1 hr, and then lipid peroxidation was measured using the reaction with TBA. The absorbance of the organic layer was measured at 532 nm. All reactions were carried out in triplicates. Vitamin C was used as a reference standard. The percentage of inhibition of lipid peroxidation was calculated, by the formula: Inhibition (%) =  $(A_{\text{control}} - A_{\text{sample}}) \times 100 / A_{\text{control}}$ .

The superoxide anion scavenging activity was determined by the method of Nishimiki *et al.* (1972). Superoxide anion derived from dissolved oxygen by a phenazine methosulfate (PMS)/NADH coupling reaction reduces nitroblue tetrazolium (NBT), which forms a violet colored complex. A decrease in color after addition of the antioxidant is a measure of its superoxide scavenging activity. To the reaction mixture containing phosphate buffer (100 mM, pH 7.4),

NBT (1 mM) solution, NADH (1 mM) and 50, 100, 150 and 200 µl of sample in 950, 900, 850 and 800 µl methanol, respectively, 1 ml of 1 mM PMS was added. After incubation at 25 °C for 5 min, the absorbance was measured at 560 nm against a blank. Vit. C was used as a reference standard.

The nitric oxide radical inhibition activity was measured by the method of Garratt (1964) using Griess reagent. Briefly, sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with 50, 100, 150 and 200 µl of sample in 950, 900, 850 and 800 µl methanol, respectively, and incubated at room temperature for 150 min followed by addition of 0.5 ml of Griess reagent (1% sulfanilamide, 2% H<sub>3</sub>PO<sub>4</sub> and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride). The absorbance of the chromophore formed was read at 546 nm.

## 2.4 Experimental protocol

Animals after acclimatization (7 days) in the animal quarters were fasted overnight and randomly divided into 4 groups of 8 animals each and treated in the following way:

Group I served as Control.

Group II Rats received physalis extract 150 mg/kg daily in drinking water orally for 12 weeks.

Group III Rats were injected with carbon tetrachloride (2mL/Kg, i.p.) once weekly for 12 weeks (Sohn *et al.*, 1991).

Group IV Rats received physalis extract 150 mg/kg daily in drinking water for 12 weeks, also, the rats were injected with carbon tetrachloride (2mL/Kg b.wt., i.p.) once weekly for 12 weeks.

Animals were killed after 24 hrs from the last dose. Blood was collected by cervical dislocation and allowed to clot for 30 min at room temperature. The serum was separated by centrifugation at 3000 rpm at 4 °C for 15 min and used for the estimation of the marker kidney function parameters namely, uric acid, urea and creatinine. The kidney were dissected out immediately, washed with ice cold saline and 10% homogenates in 50 mM Tris-HCl and 300 mM sucrose were prepared. The homogenates were centrifuged at 5000 ×g for 10 min at 4 °C and the supernatants were used for the assay of MDA, NO, GSH, SOD, CAT, GPx, GST and GR.

## 2.5 Kidney index

At the end of the experimental period, each rat was weighted. The left kidney was then removed and weighed. Finally, the kidney index was calculated by dividing left kidney weight by body weight and then multiplying it by 100.

## 2.6 Biochemical estimations

### 2.6.1 Kidney function tests

Serum uric acid (sUA), urea (sU) and serum creatinine (sCr) were assayed using kits provided from Biodiagnostic Co. (Giza, Egypt) according to the

methods described by Fossati *et al.* (1980), Fawcett and Soctt (1960) and Szasz *et al.* (1979) respectively.

#### 2.6.2 Malondialdehyde (MDA) in kidney

Levels of MDA were assayed by the method of Satoh (1978). Briefly, 0.2mL supernatant of kidney homogenate was mixed with 0.67% 2- thiobarbituric acid (TBA) and 20% trichloroacetic acid solution, and heated in a boiling water bath for 30 min. The pink-colored chromogen formed by the reaction of TBA with MDA was measured at 532 nm. The results were expressed as MDA nmol/g tissue.

#### 2.6.3 Nitric oxide (NO) in kidney

Nitric oxide level in kidney tissue homogenates was determined according to the method of Ignarro *et al.* (1987). The assay is based on the diazotization of sulfanilic acid with nitric oxide at acidic pH and subsequent coupling with N-(10-naphthyl)-ethylenediamine to yield an intensely pink colored product that is measured spectrophotometrically at 540 nm.

#### 2.6.4 Glutathione (GSH) in kidney

Levels of GSH in kidney homogenates were assayed by the method of Beutler *et al.* (1963). Briefly, the deproteinization of kidney homogenate was made by 10% trichloroacetic acid and centrifuged at 3500 rpm for 10 min. 50µl supernatant was mixed with 0.32 mol/l disodium hydrogen phosphate and 0.04% 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) solution. The yellow-colored substance formed by the reaction of GSH and DTNB was measured at 412 nm. The results were expressed as GSH mg/g tissue.

#### 2.6.5 Antioxidant enzymes in kidney

- a. Catalase (CAT) was determined spectrophotometrically by following the decomposition of H<sub>2</sub>O<sub>2</sub> in 50 mM potassium phosphate buffer, pH 7.0, at 240 nm as described by Aebi (1984).
- b. Superoxide dismutase (SOD) activity in kidney homogenate was determined according to the method of Minami and Yoshikawa (1979). This method is based on the generation of superoxide anions by pyrogallol autoxidation, detection of generated superoxide anions by nitro blue tetrazolium (NBT) formazan color development and measurement of the amount of generated superoxide anions scavenged by SOD (the inhibitory level of formazan color development).
- c. Glutathione-S-transferase (GST) activity in kidney homogenate was determined by the method described by Habig *et al.* (1974). The conjugation of 1- chloro- 2,4- dinitrobenzene (CDNB) with reduced glutathione was measured. The conjugation is accompanied by an increase in absorbance at 340 nm where the rate of increase in the absorbance was directly proportional to the GST activity in the homogenate.

- d. Glutathione reductase activity was determined spectrophotometrically as described by Bilzer *et al.* (1984). GR catalyses the reduction of glutathione (GSSG) in the presence of reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is oxidized, to NADPH<sup>+</sup>. The decrease in absorbance at 340 nm was measured.
- e. Glutathione peroxidase (GPx) was determined in kidney homogenate according to the method of Lawrence and Burk (1976). This method is based on measuring the oxidation of NADPH using hydrogen peroxide as the substrate. Absorbance was measured at 340 nm for 5 minutes, and an extinction coefficient of  $6.22 \times 10^{-3}$  was used for calculation. The results were expressed as µmol/min/gm tissue. The changes in the absorbance at 340 nm were recorded at 1-min interval for 5 min.

#### 2.6.6 Histological assessment

Kidneys from rats of different groups were fixed in 10% neutral formalin solution, dehydrated in graded alcohol and embedded in paraffin. Fine sections obtained were mounted on glass slides and counterstained with hematoxylin–eosin (H&E) for light microscopic analysis (Culling, 1974).

#### 2.6.7 Immunohistochemical analyses of Bcl-2

For immunohistochemistry, kidney section (4 µm) were deparaffined and then boiled in Declere (Cell Marque, Hot Springs, AR, USA) to unmask antigen sites; the endogenous activity of peroxidase was quenched with 0.03% H<sub>2</sub>O<sub>2</sub> in absolute methanol. Kidney sections were incubated overnight at 4 °C with a 1:200 dilution of anti Bcl-2 antibodies in phosphate buffered saline (PBS). Following removal of the primary antibodies and repetitive rinsing with PBS, slides were incubated with a 1:500 dilution of biotinylated goat anti-IgG secondary antibody. Bound antibodies were detected with avidin biotinylated peroxidase complex ABC-kit Vectastain and diaminobenzidine substrate. After appropriate washing in PBS, slides were counterstained with hematoxylin. All sections were incubated under the same conditions with the same concentration of antibodies and at the same time; so the immunostaining was comparable among the different experimental groups (Bancroft & Cook, 1994).

#### 2.6.8 Statistical analysis

The obtained data were presented as means ± standard error. One-way ANOVA was carried out, and the statistical comparisons among the groups were performed with Duncan's test using a statistical package program (SPSS version 17.0). All *P*-values are two-tailed and *P*<0.05 was considered significant for all statistical analysis in this study.

### 3. Results

Physalis extract has shown positive tests for gallic tannins, while the extract has given negative result for catechol tannins (Table 1).

**Table 1:** Quantitative analysis of tannins and its type in physalis extract.

Parameter	Amount
Gallic Tannins	+
Catechol Tannins	-

Figure 1(A) has shown the flavonoids and total polyphenolic contents of physalis extract. Flavonoids content in physalis extract was 89.4  $\mu\text{g}/\text{mg}$  quercetin

equivalents of flavonoids. The total polyphenolic content was 121.3  $\mu\text{g}/\text{mg}$  gallic acid equivalent of polyphenols.

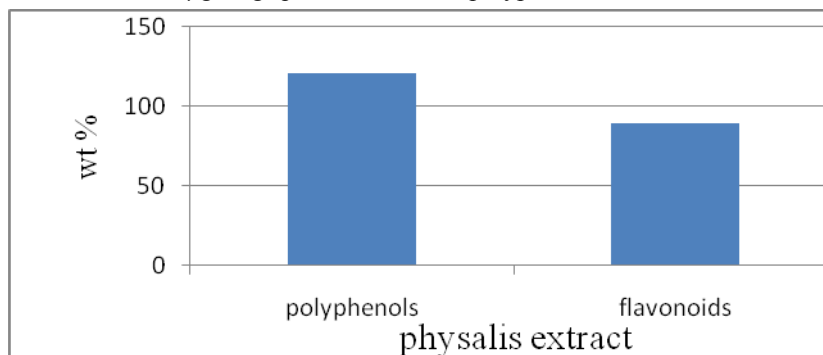


Figure 1(A): Total flavonoids and polyphenolic contents of physalis extract. (a) Flavonoids are expressed as  $\mu\text{g}/\text{mg}$  quercetin equivalents of flavonoids. (b) Total polyphenols are expressed as  $\mu\text{g}/\text{mg}$  gallic acid equivalent of polyphenols. Data are represented as mean  $\pm$  SEM of two independent experiments each performed in duplicate.

### Free Radical Scavenging Activity

As shown in Fig. 1 (B), Physalis extract revealed a concentration-dependent free radical scavenging activities resulting from reduction of DPPH and superoxide radicals. Physalis extract at concentrations

of 20-100  $\mu\text{g}/\text{ml}$ , have shown that it possesses a scavenging activities ranging from 10-100%. The scavenging activity of Vit. C, a known antioxidant, was used as positive control.

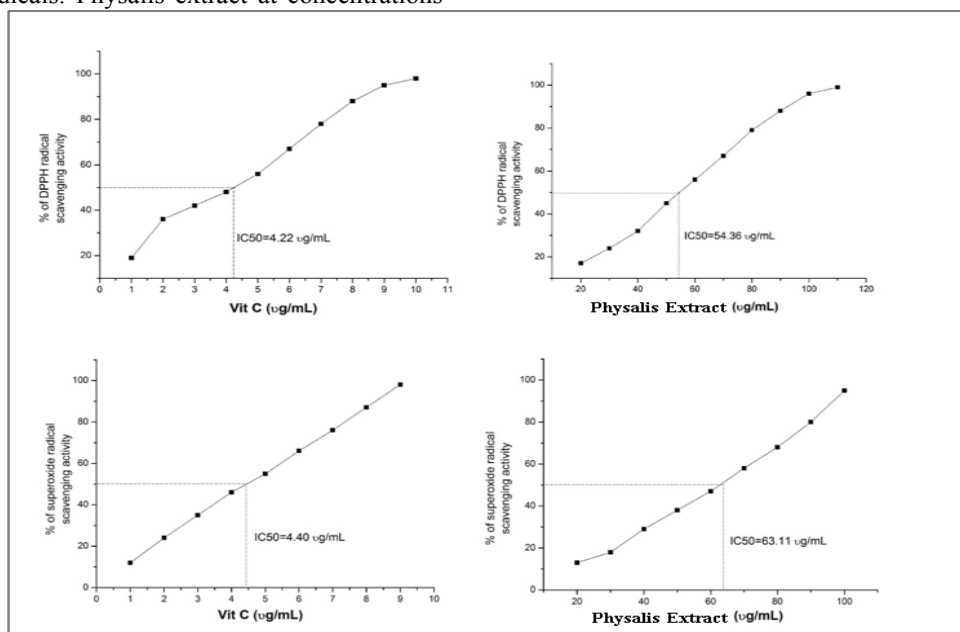


Fig. 1 (B) presents the Inhibition of DPPH and superoxide radicals by physalis extract. Data are represented as mean  $\pm$  SEM of two independent experiments each performed in duplicate.

As shown in Fig. 1 (C), Physalis extract revealed a concentration-dependent free radical scavenging activities resulting from reduction of TBARS and nitric

oxide radicals. Physalis extract at concentrations of 20-100  $\mu\text{g}/\text{ml}$ , have shown that it possesses a scavenging activities ranging from 10-100%. The scavenging

activity of Vit. C, a known antioxidant, was used as

positive control.

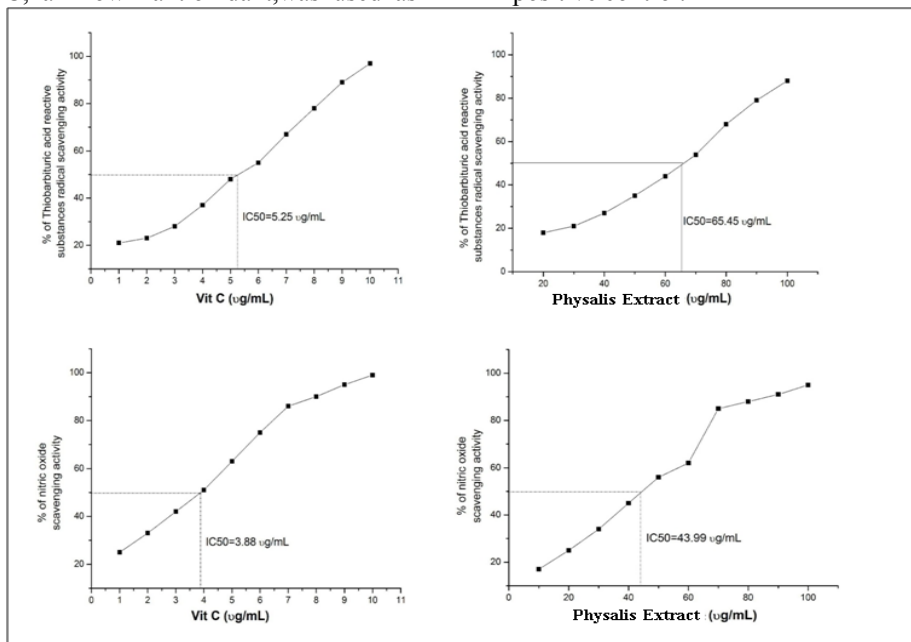


Fig. 1 (C) presents the Inhibition of TBARS and nitric oxide radicals by physalis extract. Data are represented as mean ± SEM of two independent experiments each performed in duplicate

**Effect of physalis extract and CCl<sub>4</sub> on kidney relative weight**

As shown in (Fig. 2) carbon tetrachloride induced a significant increases in kidney weight and

relative kidney weight of rats due to a significant decrease in body weight and kidney swelling . Physalis extract markedly reduced this swelling but the kidney weight was still significant increased when compared with the control group.

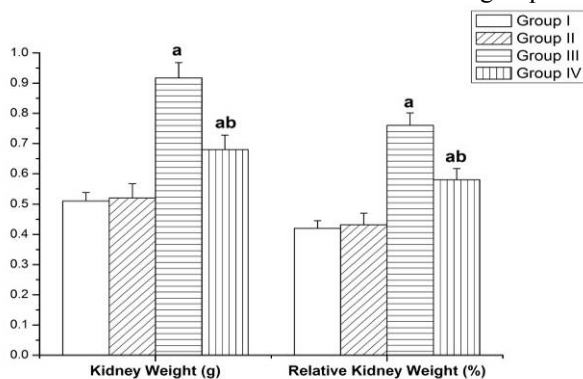


Fig. 2: Effects of physalis extract on kidney weight and relative kidney weight in rats treated with CCl<sub>4</sub>. a: Significantly different from control group. b: Significantly different from CCl<sub>4</sub> group. ANOVA followed by Duncan's test at *p* < 0.05.

**Effect of physalis extract and CCl<sub>4</sub> on kidney function tests**

The present study showed that administration of CCl<sub>4</sub> to rats caused a significant increase in creatinine (48%), urea (19.8%) and uric acid (49.51%) compared with control group (Table 2), moreover, concomitant Table (2) Effect of physalis extract on carbon tetrachloride-induced elevations of kidney function parameters of male albino rats.

administration of physalis extract and CCl<sub>4</sub> to rats caused a significant decrease by 34.26% in uric acid, 20.6% in urea and 37.83% in creatinine levels compared to CCl<sub>4</sub> group. Administration of physalis extract alone caused significant decrease in serum creatinine level by 22% versus the control group.

Groups	sU (mg/dl)	sUA (mg/dl)	sCr (mg/%)
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Group I	69.63±4.01	4.10±0.19	0.50±0.01
Group II	58.01±1.58	4.32±0.06	0.39±0.02 <sup>a</sup>
Group III	83.42±3.97 <sup>a</sup>	6.13±0.02 <sup>a</sup>	0.74±0.03 <sup>a</sup>
Group IV	65.82±1.85 <sup>b</sup>	4.03±0.16 <sup>b</sup>	0.46±0.02 <sup>b</sup>

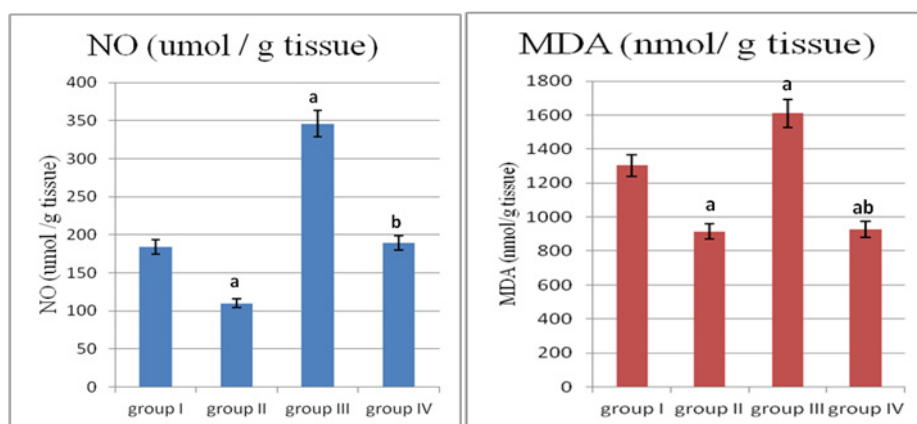
Data are expressed as means ± SEM of eight rats. **a:** Significantly different from control group at  $p < 0.05$ .

**b:** Significantly different from CCl<sub>4</sub> group at  $p < 0.05$ .

### Effect of physalis extract and CCl<sub>4</sub> on MDA and NO concentrations

The effect of physalis extract on CCl<sub>4</sub>-induced elevations of lipid peroxidation (expressed as MDA) and nitric oxide levels in the kidney tissue homogenate are shown in (Fig. 3). CCl<sub>4</sub> significantly increased the renal MDA and NO levels compared with control

group. Concomitant treatment with Physalis extract and CCl<sub>4</sub> markedly decreased MDA and NO levels compared with CCl<sub>4</sub> group. Moreover, treatment with physalis extract alone caused a significant decrease in MDA and nitric oxide concentrations in kidney tissue homogenate compared with control group.

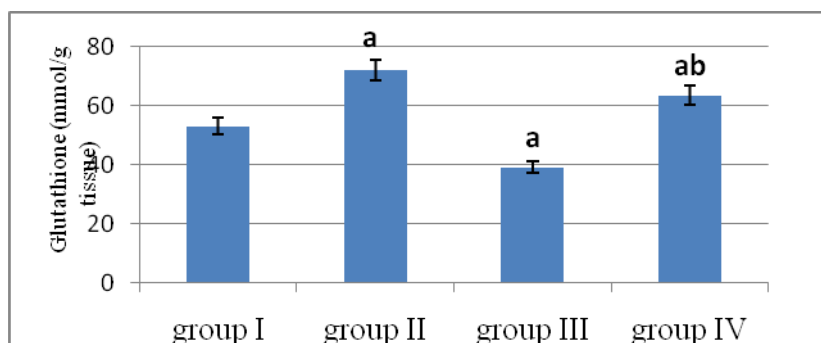


**Fig. 3:** Protective role of physalis extract on renal MDA and NO levels in rats treated with CCl<sub>4</sub>. **a:** Significantly different from control group. **b:** Significantly different from CCl<sub>4</sub> group. ANOVA followed by Duncan's test at  $p < 0.05$ .

### Effect of physalis extract and CCl<sub>4</sub> on reduced glutathione levels

Treatment with physalis caused a significant increase in GSH levels in the kidney homogenate, while treatment with CCl<sub>4</sub> induced a significant

decrease in GSH levels compared to the control group. Moreover, when rats were treated along with physalis extract and CCl<sub>4</sub> there was a significant elevation in GSH levels in kidney tissue homogenate compared to CCl<sub>4</sub> group as shown in (Fig.4).



**Fig. 4:** Protective role of physalis extract on renal GSH level in rats treated with CCl<sub>4</sub>. **a:** Significantly different from control group. **b:** Significantly different from CCl<sub>4</sub> group. ANOVA followed by Duncan's test at  $p < 0.05$ .

### Effect of physalis extract and CCl<sub>4</sub> on the activities of the antioxidant enzymes

As shown in Table (3), the activities of antioxidant enzymes were markedly decreased in

kidney tissue homogenates of rats treated with CCl<sub>4</sub> as follow: GPx (45.14%), GST (44.91%) and GR (49.18%) compared with control group. However, concomitant administration of physalis extract and



CCl<sub>4</sub> significantly attenuated the toxic effect of CCl<sub>4</sub> on the activities of the enzymes GPx, GST and GR by 85.29%, 44.17% and 113.37% respectively compared

with CCl<sub>4</sub> group. Treatment with physalis extract significantly increased the activities of GR by 19.9% and GPx by 200% compared with the control group.

Table (3) Effect of physalis extract on carbon tetrachloride-induced changes of antioxidant enzymes of kidney of male albino rats

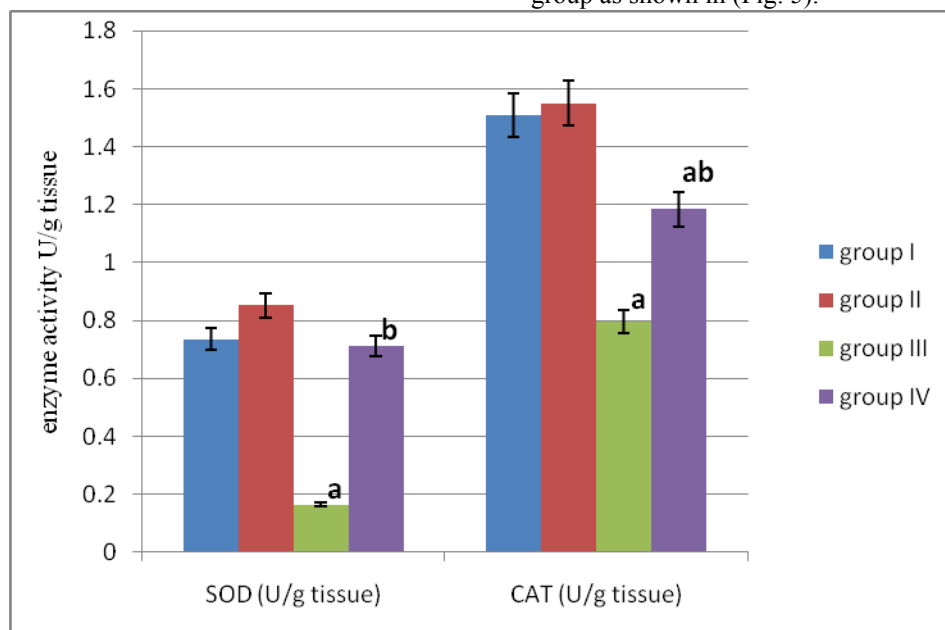
Groups	GR ( $\mu\text{mol/g}$ )	GST ( $\mu\text{mol/h/g}$ )	GPx (U/g)
Group I	158.75 $\pm$ 3.07	0.5137 $\pm$ 0.041	1125.77 $\pm$ 83.03
Group II	190.34 $\pm$ 6.74 <sup>a</sup>	0.522 $\pm$ 0.004	3377.32 $\pm$ 102.33 <sup>a</sup>
Group III	80.68 $\pm$ 7.41 <sup>a</sup>	0.283 $\pm$ 0.021 <sup>a</sup>	617.57 $\pm$ 39.86 <sup>a</sup>
Group IV	172.15 $\pm$ 10.5 <sup>b</sup>	0.408 $\pm$ 0.027 <sup>ab</sup>	1144.32 $\pm$ 66.07 <sup>b</sup>

Data are expressed as means  $\pm$  SEM of eight rats. **a:** Significantly different from control group at  $p < 0.05$ . **b:** Significantly different from CCl<sub>4</sub> group at  $p < 0.05$

#### Effect of physalis extract and CCl<sub>4</sub> on SOD and CAT activities

Treatment with CCl<sub>4</sub> markedly decreased the activities of SOD and CAT compared with the control group, while concomitant treatment with physalis extract and

CCl<sub>4</sub> significantly increased the activity of SOD as compared with CCl<sub>4</sub> group. Treatment with physalis extract and CCl<sub>4</sub> resulted in significant increase in the activity of CAT as compared with CCl<sub>4</sub> group but still significantly decreased compared with the control group as shown in (Fig. 5).

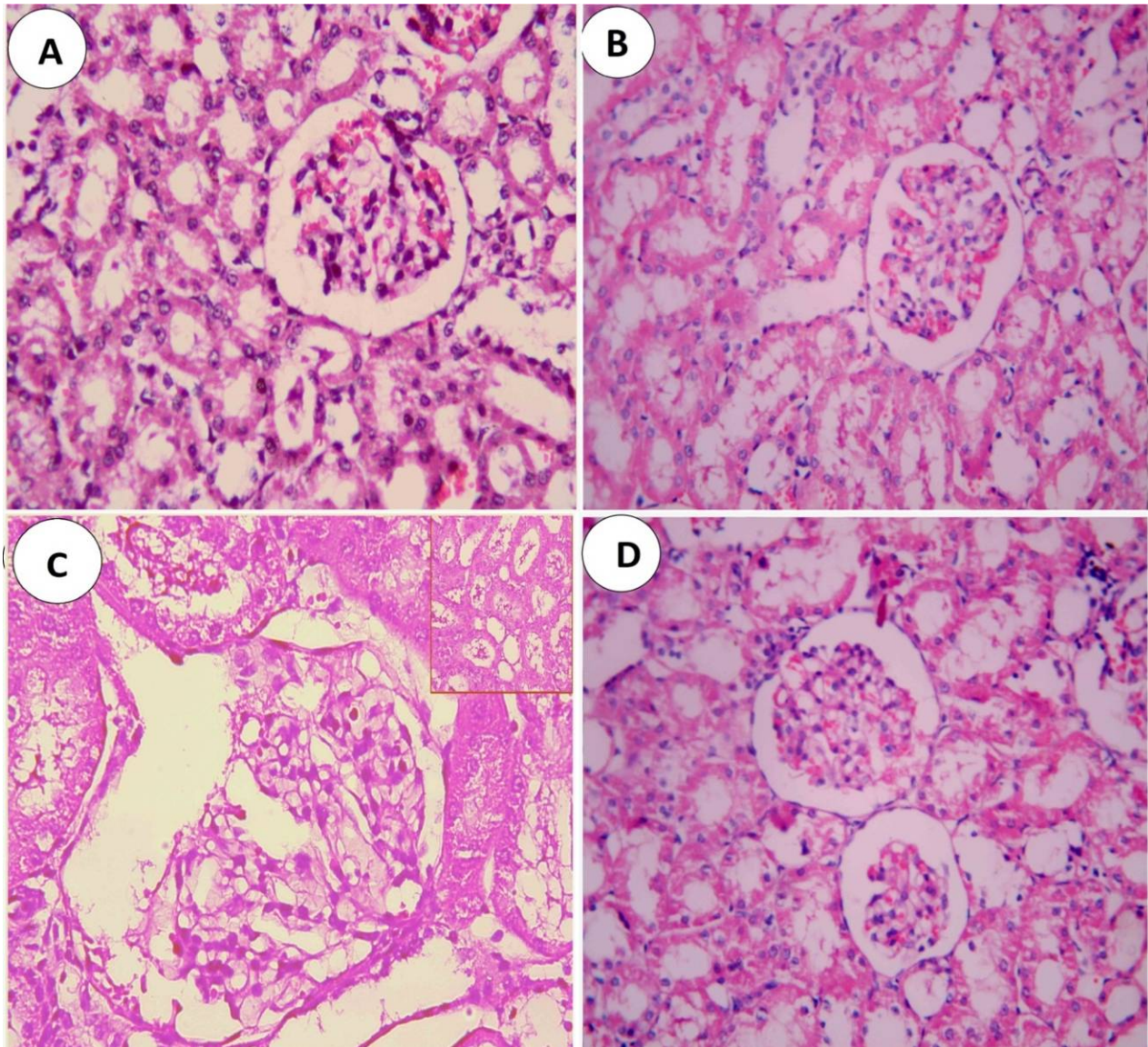


**Fig. 5:** Protective role of physalis extract on renal SOD and CAT activities in rats treated with CCl<sub>4</sub>. **a:** Significantly different from control group. **b:** Significantly different from CCl<sub>4</sub> group. ANOVA followed by Duncan's test at  $p < 0.05$ .

#### Effect of physalis extract and CCl<sub>4</sub> on histopathological changes in the kidney

There was no abnormal appearance or histological changes in the kidney of the control rats or in physalis extract treated rats, where there are normal proximal and distal tubules and intact glomerular tufts (Figs. 6A & 6B). CCl<sub>4</sub> injection caused classical damage in the rat kidney after 12 weeks, as demonstrated by congested and swollen glomeruli,

decrease in the height of epithelial cells lining convoluted tubules with vacuolated cytoplasm and pyknotic nuclei, shedding of atypical cytoplasm and loss of brush boarder (Fig. 6C). Lumen wide opens and marked congestion in vessels were also seen. Treatment with physalis extract markedly prevented congestion in glomeruli and vessels and other alterations (Fig. 6D).

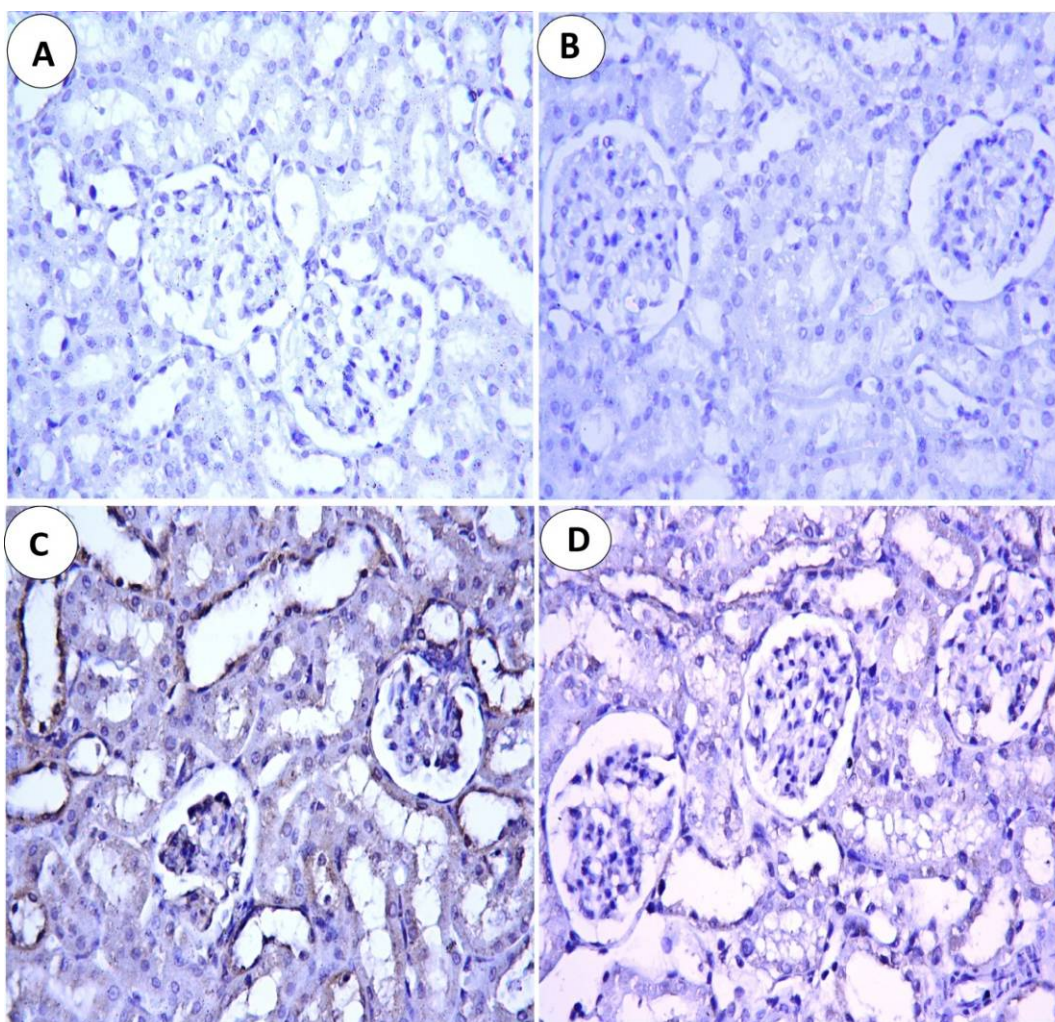


**Fig. 6:** Effect of physalis extract on histopathological damages induced by  $\text{CCl}_4$  on the kidney of rats. Kidney sections were stained using the hematoxylin–eosin method. (A) Control,(B) Physalis extract.(C)  $\text{CCl}_4$  and (D) Physalis extract and  $\text{CCl}_4$ . Original magnifications 400 $\times$ .

#### **Effect of physalis extract and $\text{CCl}_4$ on immunostaining activity for Bcl-2**

Immunohistochemical investigation for Bcl-2 showed that there was some immuno reactivity on the kidney indicating the normal life cycle of cells (Fig. 7). The immunostaining activity for Bcl-2 was decreased

in  $\text{CCl}_4$  group indicating the apoptotic effect of  $\text{CCl}_4$ . The protective effect of physalis extract was shown when rats treated with the extract where the numbers of Bcl-2 immunostaining cells were increased indicating the anti-apoptotic effect of physalis extract.



**Figure 7:** Immunohistochemical localization of Bcl-2 antigen in the kidney tissue of rats. (A) Control, (B) Physalis extract. (C) CCl<sub>4</sub> and (D) Physalis extract and CCl<sub>4</sub>. Original magnifications 400×.

#### 4. Discussion

There are various chemicals (xenobiotics) and drugs which cause damage to renal tissues by reactive oxygen species (ROS) production. CCl<sub>4</sub> is known to induce ROS, deplete antioxidant defenses; enzymatic and non-enzymatic substrates; to cause oxidative stress in renal tissues. It was reported that CCl<sub>4</sub> metabolized by cytochrome p- 450 generates a highly reactive free radical, and initiates lipid peroxidation of the cell membrane of the endoplasmic reticulum and causes a chain reaction. These reactive oxygen species can cause oxidative damage in DNA, proteins and lipids (Melin *et al.*, 2000). Various studies have demonstrated that CCl<sub>4</sub> causes free radical generation in many tissues including kidney. Olagunjua *et al.* (2009) suggested a role for reactive oxygen metabolites as one of the postulated mechanisms in the pathogenesis of CCl<sub>4</sub> nephrotoxicity.

It has also been reported that systemically administered CCl<sub>4</sub> in rats was distributed at higher concentrations in the kidney than in the liver (Sanzgiri *et al.*, 1997). Since the kidney has high affinity for CCl<sub>4</sub> (Abraham *et al.*, 1999) and contains cytochrome P450 predominantly in the cortex (Rush *et al.*, 1984; Ronis *et al.*, 1998), CCl<sub>4</sub> is extensively metabolized in the kidney generating more reactive metabolites.

The increment in lipid peroxidation as assessed by the elevated levels of MDA following CCl<sub>4</sub> administration has been well documented in kidneys (Khan *et al.*, 2009). This may be the consequence of an increment in the formation of oxygen free radicals (generated by CCl<sub>4</sub>) since antioxidant defense systems are compromised (Priscilla and Prince, 2009).

It has been hypothesized that physalis extract affords protection by impairing CCl<sub>4</sub> mediated lipid peroxidation, through decreased production of free radical derivatives. The antioxidant effect of flavonoids

that was found in physalis enhanced the process of regeneration. This might be due to destruction of free radicals, supplying a competitive substrate for unsaturated lipids in the membrane and/or accelerating the repair mechanism of damaged cell membrane.

The present study has shown that NO level significantly increased in CCl<sub>4</sub> treated animals. It has been reported that elevated levels of lipid peroxidation stimulates host cells, mainly monocytes/ macrophages, to produce and release NO by induction of inducible nitric oxide synthase (iNOS) protein, resulting in cytotoxicity and DNA damage (Raso *et al.*, 2001).

The NO radicals play an important role in inducing inflammatory response and their toxicity multiplies only when they react with superoxide anion (O<sub>2</sub><sup>-</sup>) radicals to form peroxynitrite that damages biomolecules such as proteins, lipids, and nucleic acids ( Gulcin *et al.*, 2002; Gouthamchandra *et al.*, 2010).

It was found that nitrite contents in renal tissues were increased in CCl<sub>4</sub>-treated rats (Khan *et al.*, 2009).

Nitrites can be converted into nitric oxide (NO) in acidic pH. Peroxynitrite anions have been generated by the reaction of nitric oxide and superoxide anions. These peroxynitrite anions oxidize biomolecules, which finally leads to lipid peroxidation and tubular damage (Muriel, 1998).

In the view of the current data, physalis extract has been found to decrease NO level in rats injected with CCl<sub>4</sub>. The inhibitory effect of physalis extract on NO may be due to the inhibition of the induction of iNOS protein/enzyme.

The results obtained in this study suggest the protective effects of *Physalis peruviana* extract against CCl<sub>4</sub>-induced oxidative stress, could be attributed to its high levels of polyphenols and other antioxidants like flavonoids. These compounds could scavenge the free radicals of CCl<sub>4</sub> generated through cytochrome P450 enzyme system thereby diminished the oxidative injuries.

With regard to the antioxidant defense system in this study, CCl<sub>4</sub> treatment significantly reduced the total contents of GSH, the activity levels of SOD, CAT, GPx, GST and GR indicating that CCl<sub>4</sub> has caused severe oxidative stress. It has been reported that SOD, CAT, GPx, GR and GST constitute a mutually supportive team of defense against ROS (Ji *et al.*, 1988). The decrease of non-enzymatic antioxidant defense is in agreement with the previous findings obtained by Khan *et al.* (2009).

GSH is involved in several defense processes against oxidative damage protects cells against free radicals, peroxides and other toxic compounds (Sies, 1999).

Indeed, glutathione depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects. It is widely known that a

deficiency of GSH within living organisms can lead to tissue disorder an injury ( Limon-Pacheco *et al.*, 2007).

GSH is used to evaluate the non-enzymatic antioxidant capacity of a tissue to prevent the damage associated to free radical production (Halliwell and Gutteridge, 2007).

Reductive dehalogenation of CCl<sub>4</sub> by the cytochrome P450 enzymes system to the highly reactive trichloromethyl (CCl<sub>3</sub>) radical initiates the process of lipid peroxidation which is considered to be the most important mechanism in the pathogenesis of renal damage induced by CCl<sub>4</sub> (Khan *et al.*, 2009). We cannot however exclude the possibility that CCl<sub>4</sub> metabolites may directly react with GSH, reducing its concentration. It was previously observed that CCl<sub>3</sub> radical can even react with sulfhydryl groups of glutathione and protein thiols to alter the redox status of cells (Sheweita *et al.*, 2001). Since GSH is considered an important defense against lipid oxidative damage in the kidneys eliminating hydrogen peroxide, peroxy and hydroxyl radicals formed during this process, therefore, GSH dependent enzymes will be affected when its level is depleted in the cells (Khan and Ahmed, 2009). It has been suggested that a decrease in the activities of primary antioxidant; SOD and CAT may be due to accumulation of reactive oxygen species. The observation that strengthens this hypothesis is that SOD activity can be inhibited by hydrogen peroxide treatment (Miguel *et al.*, 2009). The decreased activity of SOD in kidney in CCl<sub>4</sub>-treated rats may be due to the enhanced lipid peroxidation or inactivation of the antioxidative enzymes. This would cause an increased accumulation of superoxide radicals which could further stimulate lipid peroxidation. Also, it may result in less scavenging of free radicals leading to generation of other forms of carbon-, nitrogen- and oxygen-centered radicals, which could lead to the lipid peroxidation, nitric oxide formation and decrease in GSH level found in kidneys after CCl<sub>4</sub> administration.

Our results are in agreement with the results that obtained by Tirkey *et al.* (2005) who have conducted experiments to determine the effect of CCl<sub>4</sub> on the renal damages in rats.

Physalis extract recovered the activities of the antioxidant enzymes such as SOD, CAT, GST, GPx and GR in CCl<sub>4</sub>-treated rats. The protective effects of physalis extract in maintaining the GSH level towards control have increased the capacity of endogenous antioxidant defense and increased the steady state of GSH and/ or its rate of synthesis that confers enhanced protection against oxidative stress.

The presence of abnormally high levels of urea, uric acid and creatinine in serum are possible indicators of hepatic and/or kidney injuries induced through CCl<sub>4</sub> treatment (Ogeturk *et al.*, 2005a). The serum creatinine level does not rise until at least half of the

kidney nephrons are damaged or destroyed (Bhattacharya *et al.*, 2005).

Khan *et al.* (2009) reported that chronic renal injuries and urea elevations developed in rats after CCl<sub>4</sub> intoxication.

The present study revealed that administration of physalis extract significantly restored the levels of urea, uric acid and creatinine in serum. Similar investigations were also documented that different plant extracts significantly recovered the renal injuries induced through CCl<sub>4</sub> intoxication (Ogeturk *et al.*, 2005a; Khan *et al.*, 2010).

The protective effects of *Physalis peruviana* extract against the CCl<sub>4</sub>-induced renal injury could be attributed to its high levels of polyphenols and other antioxidants like flavonoids.

CCl<sub>4</sub>-treated rats have shown characteristic morphological findings such as interstitial fibrosis, glomerular and tubular degeneration, interstitial mononuclear cell infiltration. The vasoconstriction induced by CCl<sub>4</sub> produces an ischemic local environment, which leads to a number of cellular damages such as deterioration in membrane integrity. The severe changes were not observed in the groups treated with physalis extracts suggesting the protective effects of physalis in attenuating CCl<sub>4</sub>-induced morphological changes. Similar histopathological changes were observed by Ozturk *et al.* (2003) and Ogeturk *et al.* (2005a) in renal tissues of rats treated with CCl<sub>4</sub>.

It is believed that with these histopathological changes the capacity of tubular absorption may have been altered, thus bringing about functional overload of nephrons with subsequent renal dysfunction (Adewole *et al.*, 2007).

Immunohistochemical investigation has shown that the immunostaining activity for Bcl-2 was decreased in CCl<sub>4</sub> group indicating the apoptotic effect of CCl<sub>4</sub>. The protective effect of physalis extract was shown in rats treated with the extract where the numbers of Bcl-2 immunostaining cells were increased indicating the anti-apoptotic effect of physalis extract.

The regulation of apoptosis is another potential mechanism through which many agents such as flavonoids may prevent toxicity and cancer. Noteworthy, consequences from the toxin-induced excessive oxidative stress, depletion of antioxidant enzymes and induction of membrane lipid peroxidation may prompt the extrinsic or intrinsic apoptotic pathways. These pathways eventually lead to the activation of caspases pathway for apoptosis that ends up with caspase-3 activation, the real executioner of apoptosis once triggered, the active caspases initiated cell apoptosis (Guicciardi and Gores, 2005; Hassan *et al.*, 2011). Additionally, Cai *et al.* (2005) found that CCl<sub>4</sub> administration led to cytochrome c release from mitochondria after 4 h. It is well known that

cytochrome c released from mitochondria into the cytosol triggers the activation of caspase-9 and caspase-3 in the mitochondrial pathway. In view of this, we propose the involvement of the mitochondrial pathway via caspase-3 activation in CCl<sub>4</sub>-induced apoptosis.

We suggest that the induction of antioxidant enzymatic and non-enzymatic defense systems and suppression of MDA and NO by physalis extract could be effective in preventing apoptosis activation by caspase cascades triggered by CCl<sub>4</sub> which might be supported by previous finding (El-Mahdy *et al.*, 2008; Hassan *et al.*, 2011).

In the present study, the rats treated with CCl<sub>4</sub> showed a decrease in body weight and hence increased relative kidney weight. Chidambara Murthy *et al.* (2002) suggested that CCl<sub>4</sub>-induced weights loss might be due to gastrointestinal toxicity and by reduced ingestion of food.

It has been noticed that many of plants which are rich in phenolic compounds and flavonoids, are widely used as antioxidant and antimutagenic (Sivalokanathan *et al.*, 2006). Various chemical compounds like 28-hydroxywithanolide, withanolides, pygrine, kaempferol, and quercetin di- and tri-glycosides are reported to be present in *Physalis peruviana* (Arun and Asha, 2007).

The observed effects of the extract could be related to chemically defined compounds. Flavonoids show their antioxidative action through scavenging or chelating process. Phenolic content is also important because of the presence of hydroxyl groups possessing scavenging ability. It can, therefore, be speculated that the observed antioxidant effects of physalis extract could be due to the presence of flavonoids and phenolic contents.

The protective effects of physalis peruviana extract against CCl<sub>4</sub> - induced renal injury can be explained on the basis of its nutritional composition. It contains biologically active components e.g. physalins, withanolides, phytosterols and polyunsaturated fatty acids e.g. linoleic acid and oleic acid. Among its major components are high amounts of vitamins A, B and C as well as the presence of essential minerals, magnesium, calcium, potassium, sodium and phosphorus which are classified as macronutrients, while the Iron and Zinc, for example, are considered as micronutrients (Szefer & Nriagu, 2007).

According to Wu *et al.* (2005) Zinc is a mineral that acts as a nonenzymatic antioxidant, so that its consumption prevents oxidative damage of the cell.

β-carotene has antioxidant activity that deactivates free radicals generated in tissues (Castro *et al.*, 2008).

Vitamin C is an important dietary antioxidant, since it reduces the adverse effects of reactive oxygen and reactive nitrogen that can cause damage to

macromolecules such as lipids, DNA and proteins, which are related to cardiovascular disease, cancer and neurodegenerative diseases (Naidu, 2003).

In conclusion, the present results revealed that *Physalis peruviana* extract alleviates the nephrotoxicity induced by CCl<sub>4</sub> in albino rats. The protective effects of *Physalis peruviana* are performed through multiple ways. *Physalis peruviana* scavenges free radicals that are produced by CCl<sub>4</sub>, increases the activity of antioxidant-defense system and a greater susceptibility of the kidney to oxidant stress might be anticipated. Therefore, physalis extract may be used as a potential dietary antioxidant to retard aging and preventing diseases caused by ROS or ameliorating oxidative damage in tissues.

#### Conflict of interest statement

The authors declare that there are no conflicts of interest.

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#### 5. References

- Abraham, P., Wilfred, G., Cathrine, S.P. (1999): Oxidative damage to the lipids and proteins of the lungs, testis and kidney of rats during carbon tetrachloride intoxication. *Clinica Chimica Acta*, 289: 177-179.
- Adewole, S.O., Salako, A.A., Doherty, O.W., Naicker, T., (2007): Effect of melatonin on carbon tetrachloride-induced kidney injury in Wistar rats. *African Journal of Biomedical Research*, 10: 153-164.
- Aebi, H., (1984): Catalase *in vitro*. *Methods of Enzymol.*, 105: 121-126.
- Ahmad, S., Malik, A., Afza, N., & Yasmin, R. (1999). A new withanolide glycoside from *Physalis peruviana*. *Journal of Natural Products*, 62(3): 493-494.
- Arun, M., Asha, V.V., 2007. Preliminary studies on antihepatotoxic effect of *Physalis peruviana* Linn. (Solanaceae) against carbon tetrachloride induced acute liver injury in rats. *Journal of Ethnopharmacology*, 111:110-114.
- Ausseau, F., Samson, A., Aussagues, Y., Vandenberghe, I., Creancier, L., Pouny, I., Kruczynski, A., Massiot, G., Bailly, C., (2007): High-throughput bioluminescence screening of ubiquitin-proteasome pathway inhibitors from chemical and natural sources. *J Biomol Screen*, 12:106-116.
- Bancroft DJ and Cook CH (1994): *Manual of Histological Techniques and their Diagnostic Application*. Churchill Livingstone, Medical Division of Longman Group UK Limited. Pp:297-300.
- Beutler, E., Duron, O., Kelly, B.M. (1963): Improved method for the determination of blood glutathione. *J Lab Clin Med.*, 61: 882-888.
- Bhattacharya, H., Lun, L., Gomez, R. (2005): Biochemical effects to toxicity of CCl<sub>4</sub> on rosy barbs (*Puntius conchoni*). *Our Nature* 3:20-25.
- Bilzer, M., Krauth-Siegel, R.L., Schirmer, R.H., Akerboom, T.P., Sies, H., Schulz, G.E. (1984): Interaction of a glutathione S-conjugate with glutathione reductase. Kinetic and X-ray crystallographic studies. *Eur J Biochem.*, 138: 373-378.
- Burits, M., Bucar, F. (2000): Antioxidant activity of *Nigella sativa* essential oil. *Phytother Res.*, 14:323-328.
- Cai, Y., Gong, L.K., Qi, X.M., Li, X.H., Ren, J. (2005): Apoptosis initiated by carbon tetrachloride in mitochondria of rat primary cultured hepatocytes. *Acta Pharmacol Sin.*, 26:969-975.
- Castro, A., Rodriguez, L., & Vargas, E. (2008): Dry gooseberry (*Physalis peruviana* L) with pretreatment of osmotic dehydration. *Vitae - Revista de la Facultad de Química Farmacéutica*, 15(2): 226-231.
- Chidambara Murthy, K.N., Jayaprakasha, G.K., Singh, R.P., 2002. Studies on antioxidant activity of pomegranate (*Punica granatum*) peel extract using in vivo models. *J Agric Food Chem* 50:4791-4795.
- Culling C F (1974): *A Hand Book of Histopathological and Histochemical Techniques (Including Museum Techniques)* 3rd Edition pp. 361.
- Dewanto, V., Wu, X., Liu, R.H., (2002): Processed sweet corn has higher antioxidant activity. *J Agric Food Chem.*, 50: 4959-4964.
- El-Mahdy, M.A., Zhu, Q., Wang, Q.E., Wani, G., Patnaik, S., Zhao, Q., Arafat, S., Barakat, B., Mir, S.N., Wani, A.A. (2008): Naringenin protects HaCaT human keratinocytes against UVB-induced apoptosis and enhances the removal of cyclobutane pyrimidine dimers from the genome. *Photochem Photobiol.*, 84: 307-316.
- Fawcett, J.K., Scott, J.E. (1960): A rapid and precise method for the determination of urea. *J Clin Pathol.*, 13:156-159.
- Fischer, G., Ebert, G., & Lüdders, P. (2000): Provitamin A carotenoids, organic acids and ascorbic acid content of cape gooseberry (*Physalis peruviana* L.) ecotypes grown at two tropical altitudes. *Acta Horticulturae*, 531: 263-268.
- Fossati, P., Prencipe, L., Berti, G. (1980): Use of 3,5-dichloro-2-hydroxybenzenesulfonic acid/4-aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clin Chem.*, 26: 227-231.
- Garratt, C.J. (1964): Effect of Iodination on the Biological Activity of Insulin. *Nature* 201, 1324-1325.
- Gouthamchandra, K., Mahmood, R., Manjunatha, H. (2010): Free radical scavenging, antioxidant enzymes and wound healing activities of leaves extracts from

- Clerodendrum infortunatum L. Environmental Toxicology and Pharmacology 30, 11-18.
23. Guicciardi, M.E., Gores, G.J. (2005): Apoptosis: a mechanism of acute and chronic liver injury. *Gut* 54: 1024-1033.
  24. Gulcin, I., Oktay, M., Kufrevioglu, O.I., Aslan, A. (2002): Determination of antioxidant activity of lichen *Cetraria islandica* (L) Ach. *J Ethnopharmacol* 79, 325-329.
  25. Habig, W.H., Pabst, M.J., Jakoby, W.B. (1974): Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem.*, 249:7130-7139.
  26. Halliwell B (1991): The biological toxicity of free radicals and other reactive oxygen species. In *Free radicals and food additives*. Edited by: Aruoma OI, Halliwell B. Taylor:41-45.
  27. Halliwell, B., Gutteridge, J.M.C. (2007): Cellular responses to oxidative stress: adaptation, damage, repair, senescence and death. In: *Free Radicals in Biology and Medicine*. Oxford University Press Inc., Oxford., 187-267.
  28. Hassan, M.H., Edfaway, M., Mansour, A., Hamed, A.A. (2011): Antioxidant and antiapoptotic effects of capsaicin against carbon tetrachloride-induced hepatotoxicity in rats. *Toxicol Ind Health*.
  29. Ichikawa, I., Kiyama, S., Yoshioka, T. (1994): Renal antioxidant enzymes: their regulation and function. *Kidney Int.*, 45:1-9.
  30. Ignarro, L.J., Buga, G.M., Wood, K.S., Byrns, R.E., Chaudhuri, G. (1987): Endothelium-derived relaxing factor produced and released from artery and vein is nitric oxide. *Proc Natl Acad Sci USA* 84:9265-9269.
  31. Ji, L.L., Stratman, F.W., Lardy, H.A. (1988): Antioxidant enzyme systems in rat liver and skeletal muscle. Influences of selenium deficiency, chronic training, and acute exercise. *Arch Biochem Biophys*, 263:150-160.
  32. Khan, M.R., Ahmed, D. (2009): Protective effects of *Digera muricata* (L.) Mart. on testis against oxidative stress of carbon tetrachloride in rat. *Food Chem Toxicol* 47, 1393-1399.
  33. Khan, M.R., Rizvi, W., Khan, G.N., Khan, R.A., Shaheen, S. (2009): Carbon tetrachloride-induced nephrotoxicity in rats: protective role of *Digera muricata*. *J Ethnopharmacol.*, 122: 91-99.
  34. Khan, R.A., Khan, M.R., Sahreen, S., Bokhari, J. (2010): Prevention of CCl<sub>4</sub>-induced nephrotoxicity with *Sonchus asper* in rat. *Food Chem Toxicol* 48, 2469-2476.
  35. Kim HJ, Odendhal S, Bruckner JV (1990): Effect of oral dosing vehicles on the acute hepatotoxicity of carbon tetrachloride in rats. *Toxicol Appl Pharmacol*, 102:34-49.
  36. Kim, D.O., Chun, O.K., Kim, Y.J., Moon, H.Y., Lee, C.Y. (2003): Quantification of polyphenolics and their antioxidant capacity in fresh plums. *J Agric Food Chem* 51, 6509-6515.
  37. Lan, Y. H., Chang, F. R., Pan, M. J., Wu, C. C., Wu, S. J., Chen, S. L., Wang, S. S., Wu, M. J., & Wu, Y. C. (2009): New cytotoxic withanolides from *Physalis peruviana*. *Food Chemistry*, 116(2), 462-469.
  38. Lawrence, R.A., Burk, R.F. (1976): Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun* 71, 952-958.
  39. Lee, S.W., Pan, M.H., Chen, C.M., Chen, Z.T. (2008): Withangulatin I, a new cytotoxic withanolide from *Physalis angulata*. *Chem Pharm Bull (Tokyo)* 56, 234-236.
  40. Limon-Pacheco JH, Hernandez NA, Fanjul-Moles ML, Gonsebatt ME (2007): Glutathione depletion activates mitogen activated protein kinase (MAPK) pathways that display organ-specific responses and brain protection in mice. *Free Radic Biol Med*, 43:1335-1347.
  41. Melin A M, Perromat A, Deleris G. (2000): Pharmacologic application of Fourier transform IR spectroscopy: *in vivo* toxicity of carbon tetrachloride on rat liver. *Biopolymers* , 57:160- 168.
  42. Miguel, F., Augusto, A.C., Gurgueira, S.A. (2009): Effect of acute vs chronic H<sub>2</sub>O<sub>2</sub>-induced oxidative stress on antioxidant enzyme activities. *Free Radic Res* 43, 340-347.
  43. Minami, M., Yoshikawa, H. (1979): A simplified assay method of superoxide dismutase activity for clinical use. *Clin Chim Acta* 92, 337-342.
  44. Muriel, P. (1998): Nitric oxide protection of rat liver from lipid peroxidation, collagen accumulation, and liver damage induced by carbon tetrachloride. *Biochem Pharmacol* 56, 773-779.
  45. Naidu, K. (2003): Vitamin C in human health and disease is still a mystery? An overview. *Nutrition Journal*, 2(1), 1-10.
  46. Nishimiki M, Rao NA, Yagi, K (1972): The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem. Biophys. Res. Commun.*,46:849-853.
  47. Ogeturk, M., Kus, I., Colakoglu, N., Zararsiz, I., Ilhan, N., Sarsilmaz, M. (2005a): Caffeic acid phenethyl ester protects kidneys against carbon tetrachloride toxicity in rats. *J Ethnopharmacol* 197,273-280.
  48. Ogeturk, M. Kus, I. Kavakli, A. Oner, J. Kukner, A. and Sarsilmaz, M.(2005b): Reduction of carbon tetrachloride-induced nephropathy by melatonin administration, *Cell Biochem. Funct.* 23 85-92.
  49. Olagunju, J.A. Adeneyeb, A.A, Fagbohunkac, BS, Bisugac, NA, Ketikuc, AO, Benebod, AS (2009): Nephroprotective activities of the aqueous seed extract of *Carica papaya* Linn. in carbon tetrachloride induced renal injured Wistar rats: adose- and time-dependent study. *Biol. Med.*,1(1):1-19
  50. Ozturk, F., Ucar, M., Ozturk, I.C., Vardi, N., Batcioglu, K. (2003): Carbon tetrachloride induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. *Urology* 62, 353-356.
  51. Pietro, R.C., Kashima, S., Sato, D.N., Januario, A.H., Franca, S.C. (2000): In vitro antimycobacterial activities of *Physalis angulata* L. *Phytomedicine* 7, 335-338.

52. Prior R.L. (2003): Fruits and vegetables in the prevention of cellular oxidative damage. *American Journal of Clinical Nutrition*, 78:570s-578s.
52. Priscilla, D.H., Prince, P.S. (2009): Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats. *Chem Biol Interact* 179, 118-124.
53. Ramadan, M., & Morsel, J. (2003): Oil goldenberry (*Physalis peruviana* L.). *Journal of Agricultural and Food Chemistry*, 51(4), 969-974.
54. Raso GM, Meli R, Di Carlo G, *et al.* (2001): Inhibition of inducible nitric oxide synthase and cyclooxygenase-2 expression by flavonoids in macrophage J774A.1. *Life Sci*;68:921-31.
55. Raucy, J.L., Kraner, J.C. and Lasker, J.M. (1993): Bioactivation of halogenated hydrocarbons by cytochrome P450 2E1, *Crit. Rev. Toxicol.* 23 1-20.
56. Rice-Evans CA, Miller NJ (1996): Antioxidant activities of flavonoids as bioactive components of food. *Biochemical Soc Trans*, 24:790-795.
57. Ronis, M.J., Huang, J., Longo, V., Tindberg, N., Ingelman-Sundberg, M., Badger, T.M., (1998): Expression and distribution of cytochrome P450 enzymes in male rat kidney: effects of ethanol, acetone and dietary conditions. *Biochemical Pharmacology* 55, 123-129.
58. Rush, G.F., Smith, J.H., Newton, J.F., Hook, J.B. (1984): Chemically induced nephrotoxicity: role of metabolic activation. *Critical Reviews in Toxicology* 13, 99-160.
59. Sahreen, S., Khan, M.R., Khan, R.A., (2011): Estimation of flavonoids and evaluation of protective effect of *Carissa opaca* Stapf ex Haines fruit against CCl<sub>4</sub> induced nephrotoxicity in rat. *Food Chem Toxicol*.
60. Samouilidou, E.C., Grapsa, E.J., Kakavas, I., Lagouranis, A., Agrogiannis, B. (2003): Oxidative stress markers and C-reactive protein in end-stage renal failure patients on dialysis. *Int Urol Nephrol* 35, 393-397.
61. Sanzgiri, U.Y., Srivatsan, V., Muralidhara, S., Dallas, C.E., Bruckner, J.V. (1997): Uptake, distribution, and elimination of carbon tetrachloride in rat tissues following inhalation and ingestion exposures. *Toxicology and Applied Pharmacology* 143, 120-129.
62. Satoh, K. (1978): Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clin Chim Acta* 90, 37-43.
63. Sheweita, S.A., El-Gabar, M.A., Bastawy, M. (2001): Carbon tetrachloride changes the activity of cytochrome P450 system in the liver of male rats: role of antioxidants. *Toxicology* 169, 83-92.
64. Sies H (1999): Glutathione and its role in cellular functions. *Free Radic Biol Med*, 27:916-921.
65. Sivalokanathan, S., Ilayaraja, M., Balasubramanian, M.P. (2006): Antioxidant activity of *Terminalia arjuna* bark extract on N-nitrosodiethylamine induced hepatocellular carcinoma in rats. *Mol Cell Biochem* 281, 87-93.
66. Soares, M.B., Brustolim, D., Santos, L.A., Bellintani, M.C., Paiva, F.P., Ribeiro, Y.M., Tomassini, T.C., Ribeiro Dos Santos, R. (2006): Physalins B, F and G, seco-steroids purified from *Physalis angulata* L., inhibit lymphocyte function and allogeneic transplant rejection. *Int Immunopharmacol* 6, 408-414.
67. Sohn, D.H., Yun, Y.P., Park, K.S., Veech, R.L., Song, B.J., 1991. Post-translational reduction of cytochrome P450IIE by CCl<sub>4</sub>, its substrate. *Biochem Biophys Res Commun* 179, 449-454.
68. Szasz, G., Borner, U., Busch, E.W., Bablok, W. (1979): [Enzymatic assay of creatinine in serum: comparison with Jaffe methods (author's transl)]. *J Clin Chem Clin Biochem* 17, 683-687.
69. Szefer, P., & Nriagu, J. (2007): Mineral components in foods. New York: CRC Press.
70. Tirkey P, Pilkwal S, Kuhad A, Chopra K (2005): Hesperidin, a citrus bioflavonoid, decrease the oxidative stress produced by CCl<sub>4</sub> in rat liver and kidney. *BMC Pharmacol*, 5:2.
71. Tripathi, Y.B., Sharma, M. (1998): Comparison of the antioxidant action of the alcoholic extract of *Rubia cordifolia* with rubiadin. *Indian J Biochem Biophys* 35, 313-316.
72. Valenzuela, A., & Ronco, A. (2004): Fitoesteroles y fitoestanoles: aliados naturales para la proteccion de la salud cardiovascular. *Revista Chilena de Nutrición*, 21(1), 161-169.
73. Wu, S. J., Ng, L. T., Huang, Y. M., Lin, D. L., Wang, S. S., Huang, S. N., & Lin, C. C. (2005): Antioxidant activities of *Physalis peruviana*. *Biological & Pharmaceutical Bulletin*, 28(6), 963-966.
74. Wu, S. J., Ng, L. T., Lin, D. L., Huang, S. N., Wang, S. S., & Lin, C. C. (2004): *Physalis peruviana* extract induces apoptosis in human Hep G2 cells through CD95/CD95L system and the mitochondrial signaling transduction pathway. *Cancer Letters*, 215(2), 199-208.



## Understanding the factors influencing the amount of education-extension training activities of credit funds on rural women in Semnan province (Iran).

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**Abstract:** The purpose of this study was to determine the factors affecting the amount of extension - education activities influence on rural women in Semnan province. This research is considered applied-science in terms of objectives, highly-communicative in terms of controlled variable, and methodological study in terms of data collection and the type of field research. The study population included 1467 women members of rural micro-credit funds in Semnan province of Iran. Sample size was estimated based on the formula of Cochran 218. The samples were collected in three stages. In the first stage, the total number of ballots boxes were randomly selected, in the second stage, % of each Fund of the sample size was determined based on funds from members of the Statistical Society. In the third stage, specified number of boxes selected at random, were chosen. Data collection tools measures the questionnaire reliability with Cronbach's alpha value greater than 0.70 and its validity was confirmed by faculty advisors. The data analysis was performed using SPSS. Results showed that three components which are training courses to suit the needs of rural working women, working with other organizations and institutions with funds to carry out educational activities, adoption and the necessary funding from official organizations by the micro-credit Fund for Training courses are shown as the most important factors influencing the development of education-extension activities.

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**Keywords:** micro credit fund, rural women, educational activities - advocacy, Semnan Province

### Introduction:

In Asian and African countries, women produce and prepare more than 80% of the food.

In these regions and other regions of the world, women are denied of ownership or inheritance of the lands which they use; they have less access to land, credit, seeds, tools, training, technology and education as basic human rights (Johnston, 2006). This shows that deprivation in developing countries affects women more than men. Since rural women are a disadvantaged groups who are forgotten, they require special attention; to support the underprivileged class in many countries, various projects have been implemented such as micro-credit facilities that is paid to them (Linfeld and Knudsen, 2000).

The implication of micro-credit, which has thirty years of experience in the world, is to eliminate poverty and deprivation, provide small loans to low-income groups and to provide needed skills and technology for them (Rahmani Andbyly, 1380). Micro-credit includes the provision of appropriate financial services and is in the levels of poor households who do not have access to financial services augmented by conventional financial institutions (Mathison, 2003). The main features of micro-credit are: rapid and easy gaining no need to use expensive bail and no attention to profitability and credit services. (Rukn al-Din and honorary fellow, 1385).

Douglas considers micro-credit as part of the cycle of rural development with rural credit and agricultural credit with commonalities (Figure 1) and are paid through credit and financial institutions such as banks, credit unions, local , cooperative and ... (Douglas, 2003).

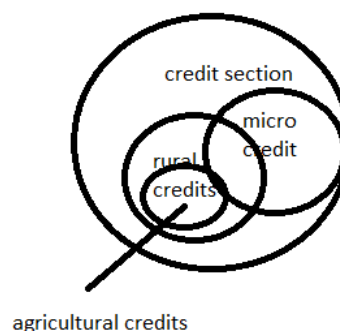


Figure 1), the position of micro-credit in the credit system

A way of escaping the trap of micro-credit intermediaries is an increase in participation and access to equal opportunities. These credits are an efficient method for empowerment, especially empowerment of

rural women, who have less access to credit sources (Seibel, 2001).

The United Nations Development Program is aimed at developing micro-credit income-generating opportunities for women, to promote investment in agricultural activities and to help farmers knowing their repayment obligations (UNDP, 1998)

ESCAP also considers the purpose of granting credit to farmers and villagers as being poverty alleviation, agricultural extension and education, employment and income generation, social development; increased self-reliance and preservation of their self-esteem (ESCAP, 1994).

According to the historical record, it is close to three decades that microfinance has become one of economic development tools and methods used for the lower strata and has become widespread in most of the countries. So that the World Bank has published a publication named "microfinance revolution". Examples of The experiences made in the field of micro-credit are founding the Grameen Bank in Bangladesh villages, and the global movement of village banks in 1976.

Nowadays, many researches about how micro-credit fund have impacted the lives of rural people have been performed. But perhaps we can find little research that directly examines the factors influencing the amount of education-extension activity - Promoting rural women's micro-credit fund. Tbrayy and Hassan Nejad (1388) research results concerning the factors influencing the adoption of the extension programs have shown that Increasing farmer age, number of children, the village of residence and employment in non-agricultural activities significantly reduces the possibility of extension programs being accepted; and variables such as having children with higher education, history of agricultural activity in wheat production, ownership of land under wheat cultivation, and the use of modern information technologies have a significant positive relationship with the probability of acceptance of the extension-education program among farmers.

Malek Mohammadi and Hossein Nia (1379 investigated the motivational factors influencing women's participation in extension programs, The findings of this study show that the most important dependent variable factors (motivation to participate) were Number of girl children, income, the amount of radio and television programs to promote attitudes, teacher attitudes, history of participation in voluntary activities, the satisfaction of the rural environment, and satisfaction with promotional programs.

Ali Beigi and Amryan (1388) in their study, considered variables such as educational level, husband's education level, age, contact with the promoters, increased production and income, and satisfaction from the training program as being influential factors in women's participation in educational- extension activities.

## Materials and Methods

This research is considered scientific study in terms of objectives and considered highly-communicative in terms of controlled variable, and the research methodology is after-the-event type, In terms of data collection is the type of field research.

The main objective was investigating the factors influencing the amount of training activities of micro credit fund for rural women in the province of Semnan. Dependent variable of this research was education-extension activities provided by micro credit Fund for rural women. And for its assessment, eight components extracted from previous studies, was used. The women were asked their opinions about the amount of demonstration available for each of the activities (components) that they are a member of, in the form of Likert, determined by very low (1) to very high (5). The total amount of the 8 components of education-extension activities was calculated by the micro-credit fund.

Also independent variables such as Marital status (nominal), age (distance), education (ordinal), occupation (nominal), the amount of monthly income (distance), a history of credit in the Fund (the distance), parity (the a), membership in the social Drnhadhay (nominal), the amount of cash credit loan (space), consumption loans (nominal), frequency of participation in training - extension (distance), assessment of the number of women education - Promoting held (sequential), Guyana on the assessment of the quality of training - extension event (sequential), offerings of training courses - Extension to other organs (nominal), Rural women's micro-credit funds in cooperation with other organizations (nominal), the members of credit to fund educational activities, and member satisfaction were among the presented activities by the micro-credit funds.

To assess members' satisfaction and points of views, the 9<sup>th</sup> and 10<sup>th</sup> components of the previous research were respectively used. The study population included all women members of rural micro-credit funds in the province that their number is about 1467 people. Cochran formula based on the sample size was estimated at about 218.

The sampling was conducted in three stages, first a number of credit funds were selected among the total number of credit funds of Semnan province, in the second stage using a stratified sampling method, in accordance with the percentage of fund members from the statistical community % Of each Fund of the sample (sample size) was determined, and then in the third step of the funds, specified samples were selected randomly.

In this research a survey questionnaire was used to collect data. And data assessment was carried out with Cronbach's alpha values higher than 70/0. And its validity was confirmed by the faculty advisors. Data

analysis using SPSS software was conducted in two parts of descriptive and inferential analysis.

## Results

### 1) characteristic of the subjects (descriptive statistics)

The results of this study showed that Many of them (81.2 percent) had received loans from the credit fund and the remainder (18.8 percent) had not yet received the loan. Of women who had received loans until this study (177 patients), the majority (74.6 percent) used their loans for non-productive activities and only 25.4% of women had used it for production activities. Women members of this funds on average had only participated 1.04 time in training – extension. Among women who had participated at least once during this period (127 people), only 27.5% of members participated in training had suggested ‘extension’ to other members. According to the majority of respondents (74.3%) of women of micro-credit fund have not worked with other organizations.

A summary of the results of the Descriptive statistics related to other variables is presented Table (1).

Table 1 :dependent variables relating to the respondents personal Characteristics

Variable(unit)	minimum	average	maximum
Age(in years)	18.	3 .53.	71.
Children(persons)	0.	3.31.	9.
Monthly income(Rls)	0.	477.064.	5.000.000.
Loan received from Fund(Rls)	1.000.000.	9.689. 65.	30.000.00.
Number of times participated in educational-extension activities	1.	01.04.	5.
Women's assessment of the number of training courses - held in extension	.1. Very low	.17.	5. Very high

### 2) prioritizing of the components affecting the development of educational - extension activities in the respondents view:

In this section, to determine the factors that influences the prioritization of development of training - extension activities in the point of view of the respondents, 13 components were given to the respondents and they were asked their views about the importance of each component on the development of training activities; the results were in the form of Likert type, from very low (1) to quite a lot (5) to express. Then prioritize was obtained regarding the change coefficient (cv).

Table (2), components of effective prioritization of development activities (training – extension) in the views of respondents:

Factors affecting the development of extension education activities	Standard deviation	Average(mean)	change coefficient(cv)	priority
Training courses to suit the business needs of rural women	6 9.0.	57.4	138.0.	1.
Working with other organizations and institutions with funds to carry out educational activities - extension	65 .0.	3 .4.	145.0.	.
Approval and the determination of funding from official organizations to hold training courses on micro-credit	650.0.	11.4	158.0.	3.
free training courses for members	677.0.	.4	160.0.	4.
Allocated loans by the Fund for courses	773.0.	6.4	181.0.	5.
Fund Authorities use of other fund of experiences in organizing training courses	869.0.	54.3.	45.0.	6.
Using all channels of communication extension to encourage rural women to participate in this training	838.0.	18.3.	64.0.	7.
Training courses that suits the literacy level and knowledge of rural women	885.0.	34.3.	65.0.	8.
Organizers have a positive attitude to the efficiency and effectiveness of training courses	773.0.	87. .	69.0.	9.
Using posters, educational and promotional publications related to rural women's entrepreneurship	6 4.0.	49.1.	419.0.	10.
Planning training courses for rural women who have free time	895.0.	90.1.	471.0.	11.
Timely and sufficient information for members to attend training courses - extension	15 .1.	11. .	546.0.	1 .
Granting the relevant documents to the participants in this training	149.1.	01. .	57 .0.	13.

Scale: low (1), very low (2), medium (3), high (4), very high (5)

As it is shown in table 2, in the view point of respondents, three components which are training courses to suit the needs of rural working women, working with other organizations and institutions with funds to carry out educational activities, adoption and the necessary funding from official organizations by the micro-credit Fund for Training courses are shown as the most important factors influencing the development of education-extension activities.

Also the three components of granting the relevant documents to the participants in training, planning,

organizing training courses for rural women having free time and providing information in a timely and sufficient manner to members who attend training courses, were considered by the respondents, the least important component in the development of educational- extension activities.

### Conclusion

The survey results showed that factors affecting the development of extension-educational activities of micro credit fund in the province were categorized in five components. The extracted factors will be discussed in order of importance:

1. Fund support from government agencies  
First and third buoy in the prioritization factors were taken categorized as the second and third important variables. These results represent an important role in supporting government agencies from the fund.

Since these funds are formed with little capital and cannot have high financial capability, often lack sufficient resources to carry out educational activities and on the other hand, micro-credit fund can achieve its predetermined objectives if they do provide education - extension activities to their members. Therefore, it is essential to work through and with other organizations for providing support for such activities; otherwise, micro credit funds alone will not be able to provide educational activities with successful act.

2. Encourage members to participate in education- extension activities

Measures that can be taken on encouraging members to participate in educational activities and promoting the development of these activities are the buoy that have been included in this factor.

For example, the granting of the relevant documents to the participants in this training could encourage more members to participate more in the extension educational activities. Using qualified personnel and promoting quality of teachings in order to increase the quality of these courses can therefore be welcomed by most members of the training.

Also, because most members do not have a good financial situation free training for them can also be a factor in encouraging them to attend the educational activities. A Noteworthy

point is to increase the participation of members in educational activities with mandatory methodology, just like the Fund's loans to the members, may only increase participation in cross-sectional levels but cannot motivate and change their attitude towards training activities.

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### Resources:

1. Anonymous, (1390) Introduction to micro-credit fund Monthly Research, Education and Extension Dhyary News, No. 33, pp. 30-25.
2. Tbrayy, M. and M. Hassan Nejad, 1388, yield and factors affecting the adoption of extension programs implemented in the process of agricultural development: case study of wheat growers in Mashhad city, Journal of Economics and Agricultural Development (Science, Industry and Agriculture), Volume 23, Number 1, first semester 1388, pp. 68-59.
9. Najafi, Bhaaldyn and J. James, (1384), micro-finance: a new mechanism for reducing poverty in rural communities, agricultural and development economics, year XIII, Number 49, Spring 1384.
10. Ali Beigi, AJ and Leila Amryan (1388), and demographic factors affecting the participation of rural women in extension programs: case study and Klyayy falcon city, socio-psychological studies of women, age 7, Number 2, Fall 1388, pp. 133-115.
11. Koushki, F., H. Iravani and Khalil Kalantari, 1390, Factors affecting women's participation in rural micro-credit fund: a case study in Kermanshah Province, Journal of Rural Development, Year 14, Number 1, Spring 1390, pp. 33-15.
12. A. Malek Mohammadi. Nia H. Gh. H., 1379, rural women's participation in educational programs and promotional incentives in Fars Province, Science, Agriculture, Iran, 31: 54-39.
13. Mirbagheri, Akram Sadat, in 1385, social and cultural factors affecting rural women's tendency to engage in micro-credit fund: a case study in Mazandaran province, M.Sc. Thesis, Tarbiat Moalem University, Tehran
14. FAO / ALIDE (1996), Collateral in rural loans, October 1996, Association of Latin American Food and Agriculture Organization (FAO) and

development finance institutions of the United Nations, (ALIDE)

15. Gotez, Anne Maria and Ran Sen Gupta (1994), Who takes the credit? Gender, power and control over loan use in rural credit program in Bangladesh Brighton. England Institute for Development studies, university of Sussex.

16. Seibel (2001), Rural finance for the poor: from unsustainable projects to sustainable institutions, IFAD (Rome 2001). [www.unikoeln.de/ew-fak/aef/05-2004/2001-](http://www.unikoeln.de/ew-fak/aef/05-2004/2001-6%20Mainstreaming%20Informal%20FIs.pdf)

[6%20Mainstreaming%20Informal %20FIs.pdf](http://www.unikoeln.de/ew-fak/aef/05-2004/2001-6%20Mainstreaming%20Informal%20FIs.pdf)

17. Shivads, Meena. (2004). A short note on micro credit. International seminar on micro credit for youth, Shiaz Iran.
18. UNDP (1998), Microfinance institutions proposal: Executive summary, port Moresby.
19. Zvi, L. (2001), Productivity and efficiency of individual farms in Poland: a case for land consolidation, Department of Agricultural Economics and Management, The Hebrew University, Rehovot, Israel ([lerman@agri.huji.ac.il](mailto:lerman@agri.huji.ac.il)), May 2002.

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**Mentha piperita and Depressive disorders: A controlled trial**

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**Introduction:** Depressive disorders are among the most important disabling diseases that have affected a large population throughout the world. This study has been carried out with the aim to determine the effect of Mentha piperita on improving the symptoms in persons with depression as a co treatment. **Methods:** The effect of Mentha piperita on depression was studied in a triple-blind random clinical trial. The applied tool was Beck questionnaire and the number of samples was 55. Collected data were analyzed using SPSS software taking the help of descriptive statistics, one-way Variance analysis and Variance analysis with repeated measurements. **Results:** The results of the research showed that, there was a statistical significant difference at the level of  $p=0.01$  among under studied groups in such a way that's, Mentha piperita reduced the symptoms of disease and improved the under research samples. **Conclusion:** Some plants could be used as treatment complementary in psychical disorders therefore; the necessity of more researches is felt in this regard.

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**Keywords:** Depression, Mentha Piperita, Complementary.

**Introduction:**

Depressive disorder is considered as a common disorder distributed in the population, and usually associated with substantial symptom severity and role impairment(1). Being female, middle-aged, widowed, separated, or divorced, and low income can increased risk of depression. Men were significantly less likely to receive treatment than women(2). The lifetime prevalence of depressive disorder varied from 6.9% to 16.2 % (1-4). Sign and symptoms of depression are depressed mood, anhedonia, decreased energy, difficulty in concentration, insomnia, anorexia, helplessness, hopelessness, guilt feeling, and suicidal thoughts or death. Depression usually results in difficulty in occupation and social function and interpersonal relationship. More than 2/3 had suicidal thought and finally 15% of the depressed patients commit suicide(5). Physical manifestation of depression as a prevalent psychological disease usually causes lack of correct recognition of depression in patients and such problems exist in all cultures(6,7). At present, several pharmacologic and non-pharmacologic treatments are performed for depressive disorder. Epidemiologic researches suggest that patients turn to dietary supplements because of a reluctance to take prescription medications or a lack of satisfaction with the results. They often perceive

dietary supplements to be a safer and more natural alternative. Patients with mental health disorders, including depression, are among those who use dietary supplements(8). Today, more and more individuals use complementary/ alternative therapies for treatment of physical and mental disorders. Eisenberg et al (1993) conducted a national survey in the US, finding that one in three respondents had used at least one alternative therapy in the last year(9). There is a lot of researches on effect of herb on psychological and physical disorder for example: Peppermint was found to enhance memory, increased alertness, but Yalang-Yalang decreased it (10), the beneficial effect of FEWP(Free and Easy Wander Plus) for mood disorder(11), the role of essential oils in the treatment of Attention Deficit Hyperactivity Disorder (ADHD)(12), the use of hypericum perforatum (St Johnson's wort) to treat depression, Ginkgo biloba to delay cognitive decline in patients with Alzheimer's disease(13), the effect of the herb on insomnia (14), the effect of odor compounds of Mint, Ostokhodoos and Rosemary on the stress of nursing students, indicated that depression and stress of the students have been reduced(15), effect of peppermint oil in irritable bowel syndrome(16), the other plants with CNS-effect used (17) and etc. All of the researches were mixed of some plants and M. Piperita is one of

them. Mint is a grassy, nutrient vegetable. It uses as a complementary food by people, the same as lettuce or parsley. It is cultivated in most temperate zones of the world. The present study was done in order to determine the effect of the pure extract of Mint on the depressive disorder.

#### Methods:

This research is a double blinded randomized controlled parallel trial to measure the effect of mint extraction on the treatment of severe depression. The samples consisted of the depressed patients referred to Shahid Mofateh Clinic of Yasouj City (south of Iran) during 2007-2008. The inclusion criteria were the age limit of 18-60, having depressive symptoms for at least two weeks, receiving similar treatment regime, having no other psychological disorders, no history of admission in psychiatry ward, no ECT (electroconvulsive therapy) and no history of taking any drug except antidepressant drugs ordered by their psychiatrist. One hundred twenty patients were selected at the starting point of the research. Patients were randomly allocated to five groups; placebo (control group), and four experimental groups, namely, those received daily 10.0 mg, 50.0 mg, 100.0 mg or 200.0 mg of hydro alcoholic extract of *Mentha Piperita* for two months. Each group consisted of 24 patients. Severity of depression was measured by a psychiatrist, using BDI (Beck Depression Inventory) and DSM IV-TR criteria before and 2 months after intervention. To prepare the extract, in September the time of flowering of Mint, the fresh mint plant samples were collected. The samples were cleaned, washed and dried in shadow. Dried Mints were then grounded and soaked in water and alcohol with the proportion of 50: 50 for a period of 48.0 hours and then filtered. Thereafter, water and alcohol were separated using rotary machine(18). Then, the extract was injected in capsules with rates of 10.0 mg, 50.0 mg, 100.0 mg and 200.0 mg and packed Placebo was prepared using the same capsules. These packages were kept in refrigerator before being delivered to the patients. The study was double blinded in such a way that the researcher was aware of the grouping but the co-researcher who had the responsibility of collecting data along with the psychiatrist, the psychiatrist who was responsible for diagnosis and the patients were unaware of the study grouping. Researchers did not find any significant side effect for mint extract. This research was carried out by a financial support from

the Vice Chancellor for Research of Yasouj University of Medical Sciences. The trial registration number is: 201011105147 N1. Informed consent was also obtained from the patients. The usual anti depressant treatment of all groups of the study was continuing throughout the research period. Fifty four percent of the participants were lost to follow up (39% loss of those who were not continue to the research and eliminating 15% of persons whose data was incomplete). The main reason for such a significant loss was a sudden and huge increase in the fuel cost in Iran (7 times higher than the time when the study was started). The high cost in transport as well as the fact that most participants were from remote rural areas with a low socio-economic background could have prevented a significant number of the participants to attend the follow-up procedures including post test. The losses to follow rates were similar in all study groups (13, 15, 12, 11 and 14 for placebo, 10, 50, 100 and 200 groups respectively). Accordingly, the statistical analysis was carried out on the data from 55 persons. The collected data were analyzed using Chi-Square test and one way ANOVA for repeated measures. The power of the statistical analysis was calculated at about 60%.

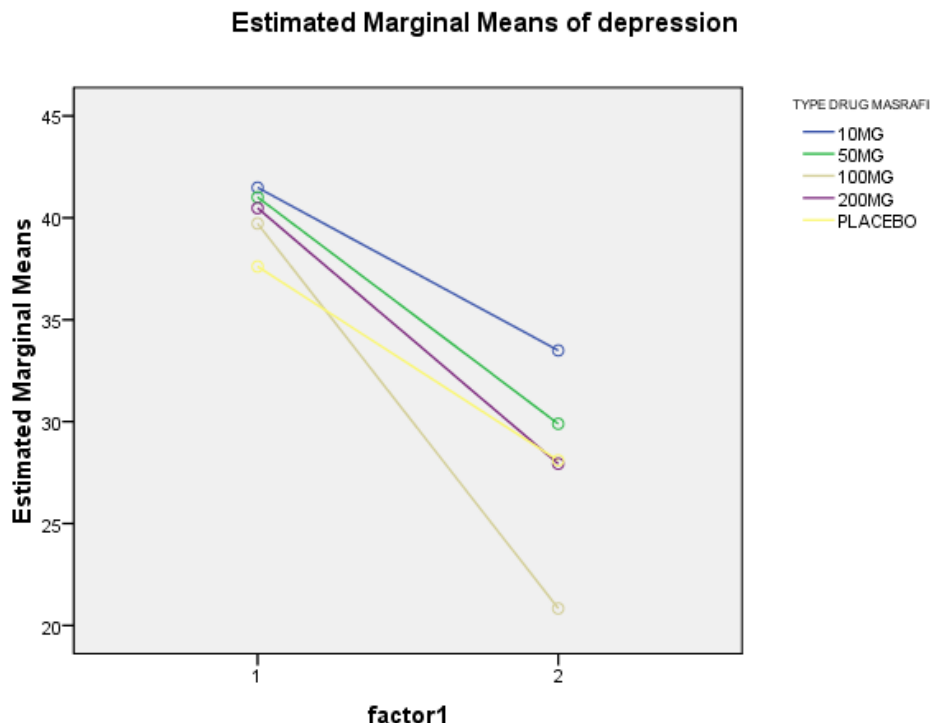
#### Results:

In total 55 participated in this study. Patients were divided into five groups by randomized allocation method. Majority of the experimental groups (68.2%) and control group (54.5%) were women. The mean age of those in the case groups was  $27.9 \pm 8.5$  (with a range of 18-52 years) and in control groups was  $29.3 \pm 13.9$  (with a range of 18-60 years). In the experiment groups, 24 patients (54.5%) were married comparing to (54.5%) in the control group. One way ANOVA & Chi-Square Test show no significant difference between age, sex, marriage situation, and familial history of depression disease, level of education and occupation status of patients in both experiment and control groups. Table 1 shows the distribution and the comparison of the mean and ST-deviation of depression in case and control groups before and after intervention. The highest anti-depressant effect was seen in those patients who received 100 mg and 200 mg of the extract respectively and the lowest score belongs to the groups of 50 mg, placebo group and 10 mg, respectively ( $P < 0.01$ ). According to Shefe procedure the difference was significant between 10 milligrams group and 100 milligrams group.

**Table 1:** The mean and standard deviation of depression in the groups under the study before and after intervention

Group Time	Control (No=11.0)	10 mg (No=9.0)	50 mg (No=12.0)	100 mg (No=13.0)	200 mg (No=10.0)	Significant level
Before intervention	36.7±6	40.1±8.3	40.2±8.8	38.9±10.6	40.6±5.3	NS*
After intervention	26.6±9.7	34±7.8	29.4±9.6	20.1±7.5	28.4±10.2	P<0.01

\* NS= Not Significant



### Discussion & Conclusion:

Depression is a common psychiatric disease, which is among the most disabling health and psychological problems in different societies. Depressive disorder is associated with role impairment, difficulty in social and occupational function and the patient may commit suicide (1-5). At present, several pharmacologic and non-pharmacologic treatments have been applied for this disease mostly expensive with a large number of unwanted complications. Psychological therapy is also time consuming. Accordingly, using herbs as treatment or as a complementary treatment can be valuable. This study was done in order to determine the effect of mint extract on depressive disorder.

It was revealed in this study that the severity of depression in the experimental groups improved significantly. Based on the results of this research, the Mint extract at the rates of 100.0 mg and 200.0 mg was more effective on the patient's disease status. Several studies on the effect of herbal medicine on different diseases and disorders showed positive effects of herbs on memory, alertness and diseases like IBS, ADHD and stress (17-23).

According to the results of this study, mint may be used in treatment of depression, particularly in 100 mg and 200 mg per day. It wasn't possible to find out the effective ingredient of the extract; however, based

on the results of a published study Narigenin may be the effective content of mint extract(19). With no or very limited side effects we suggest M. Piperita may be used as a complementary regimen in control of depressive disorder. Obviously, further research is needed to find out the most effective content and the mechanism of its effects on depressive disorder.

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### Mention:

The Farsi version of the results of this study is published in Armaghan Danesh, a local journal of Yasouj university of medical sciences and it's abstract has been indexed by Index Copernicus, Google scholar, IMEMR & ISC.

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### References:

1. Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Kathleen RM, Rush J, Ellen EW, Philip SW.



- The epidemiology of major depressive disorder. *JAMA* 2003; 289 (23):3095-3105.
2. Deboreh SH, Renee DG, Fredrick SS, Bridget FG. The epidemiology of major depressive disorder. *Arch Gen Psychiatry* 2005; 62:1097-1106.
  3. Kessler RC, Chiu WT, Demler O, Walters EE. Prevalence severity and morbidity of twelve months DSM-IV disorders in the national comorbidity survey replication. *Arch Gen Psychiatry* 2005; 62 (6): 617-27.
  4. Takeuchi DT, Chung RC, Lin KM, Shen H, Kurasaki K, Chun CA, Sue S. Life time and twelve month prevalence rate of major depressive disorder and Dysthymia among Chinese Americans Los Angeles. *Am J Psychiatry* 1998; 155(10): 1407-14.
  5. Kaplan HI, Sadock BJ. Synopsis of psychiatry: behavioral science, clinical psychiatry. 10<sup>th</sup> ed. 2007 by Lippincott Williams & Wilkins, Philadelphia. pp: 534-58.
  6. Okulate GT, Olayinka MO, Jones OB. Somatic symptoms in depression: evaluation of their diagnostic weight in an African setting. *Br J Psychiatry* 2004; 184: 422-7.
  7. Tylee A, Gandhi P. The importance of somatic symptoms in depression in primary care. *Prime care companion J Clin Psychiatry* 2005; 7(4): 167-176.
  8. Cauffield JS, Forbes HJ. Dietary supplements used in the treatment of depression, anxiety and sleep disorder. *Lippincotts Prim Care Pract* 1999; 3(3): 290-304.
  9. Jeffrey R. Flower essence therapy in the treatment of major depression: preliminary findings. *The International Journal of Healing and Caring* 2001; 1(1): 1-16.
  10. Moss M, Hewwitt S, Moss L, Wesnes K. Modulation of cognitive performance and mood by Aromas of Peppermint and Yalang-Yalang. *international journal of neurosciences* 2008; 118(1): 59-77.
  11. Li LT, Wang SH, Ge HY, Chen J, Yye SW, Yu M. The beneficial effects of the herbal medicine Free and Easy Wanderer Plus (FEWP) and Fluoxetine for mood disorders: Double-blind, placebo-controlled studies. *the Journal of Alternative and Complementary Medicine* 2008; 14(7) :841-6.
  12. Godfrey H. The role of essential oils in the treatment and management of attention deficit hyperactive disorder (ADHD). *International Journal of Aromatherapy* 2001; 11(4): 193-200.
  13. Richard LN, Stephen ES. Research into complementary and alternative medicine: problems and potential. *BMJ* 2001; 322: 161-164.
  14. Norrish MIK, Dwyer KL. Preliminary investigation of the effect peppermint oil on an objective measure of daytime sleepiness. *International Journal of Psychophysiology* 2005; 55(3): 291-8.
  15. Park MK, Lee ES. The effect of aroma inhalation method on stress responses of nursing students. *Taehan Kanho Hakhoe Chi* 2004; 34(2): 344-51.
  16. Cappello G, Spezzaferro M, Grossi L, Manzoli L, Marzio L. Peppermint oil (Mintoil) in the treatment of irritable bowel syndrome: a prospective double blind placebo-controlled randomized trial. *Digestive and liver disease*. 2007;39(6): 530-536.
  17. Gary IS, Mikael EP, Johannes VS, and Anna KJ. Review on plants with CNS effects used in traditional South African medicine against mental diseases. *Journal of Ethno pharmacology* 2008; 119 (3): 513 -537.
  18. Sadeghi H, Yazdanparast R. Effect of *Dendrostellera lessertii* on the intracellular alkaline phosphatase activity of four human cell lines. *Journal of Ethno pharmacology* 2003; 8:11-14.
  19. Oslen T, Stafford g, Staden J, Soren B, Jager C, Jager K. Isolation of the MAO-inhibitor naringenin from *Mentha aquatica* L. *Journal of Ethno pharmacology* 2008; 117(3),500-502.

7/1/2012

## Nurses' Practice, Knowledge and Attitude towards Evidence-Based Practice at Yanbu general hospital –kingdom of Saudi Arabia

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**Abstract:** More than 25 years have already passed since research utilization has been discussed in the nursing literature with enhanced enthusiasm and demands for using research findings into practice. Moreover, the movement of evidence-based practice which started in 1990s has underlined the significance of integrating research utilization in practice. The aim of this work was to assess the knowledge, attitude and practice of nurses towards evidence based practice and factors influencing them. Methodology: A descriptive cross sectional study, carried out at Yanbu General Hospital in kingdom of Saudi Arabia. collection of data was carried out over a period of 3 months. Results: Study was carried out on 156 nurses, the response rate was 52%, participants below 30 years old were 134 (85.9%), those 30 – 40 years old were 16 (10.3%) and those above 40 years old were 6 (3.8%). They were 145 (92.9%) female nurses and 11(7.1%) male, the majority 120 (77%) were registered nurses and the majority 103 (66%) were earned diploma of nursing as their highest education, Most of them 113 (72.4%) work for more than 40 hours per week, the majority 124 (79.1%) have less than 5 years of experience. Studying factors influencing KAP of EBP among nurses revealed non significant difference between nurses either due to educational level or nationality neither in knowledge of terms related E.B.P., Attitude toward E.B.P. and practice of E.B.P. between bachelor earned and diploma, no difference between Saudian and non Saudian nurses as regards knowledge related E.B.P. Whereas Attitude of Saudian nurses toward E.B.P. shows significantly lower percentage than non Saudian, also practice of E.B.P. shows significantly higher percent of non Saudian (60.5%) their sum of self reported practice was always, versus 39.5% of Saudian. Age group difference for knowledge about E.B.P. related terms shows significantly increase percent of knowledge. Attitude towards E.B.P. significantly increase with age. Although self reported practice increase with age yet no significant difference observed.

Conclusion: The observed lack of the required knowledge and practice of evidence based practice among nurses in the current study raise the issue of applying evidence based practice in hospitals and other health care facilities and assessing that during reviewing the health institution for renewal of license or accreditation.

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**Key words:** nursing evidence-based practice, attitude, and knowledge

### 1. Introduction

Evidence-based practice is defined as the integration of best research evidence with clinical expertise and patient values and the conscientious, explicit, and judicious use of such evidence in making decisions about the care of individual patients. (1)

Evidence-based practice in nursing (EBP) has been presented as a decision-making model or a model for solving clinical problems(2). The basic steps involved in evidence-based nursing practice are defining a problem and formulating clinical questions that can be answered through research or other sources of evidence, finding the best evidence to answer these clinical questions, assessing the validity of the evidence to provide answers to clinical questions, incorporating the evidence with nurse's expertise and patient's attitude and finally evaluating the whole process and the results (3).

In the United States of America, the Institute of Medicine, American Nurses Credentialing Center (ANCC), and the Joint Commission on Accreditation of Healthcare Organizations recognize evidence-based practice as a critical step in improving healthcare quality. The Institute of Medicine recognizes evidence-based practice as an essential competency for healthcare providers in the 21<sup>st</sup> century (4) The reasons why nurses should integrate research findings in their routine practice are several.

Utilization of research findings increases the quality of health care, provides increased efficiency in patient care and professional attitudes of the nurses are developed and Nursing research produces knowledge that nurses can use in their routine work (5,6) The Joint Commission has consistently supported the implementation of evidence-based practice in nursing as a means of improving healthcare systems,

Evidence-based practice has repeatedly been shown to improve patient outcomes. The Joint Commission has consistently supported the implementation of evidence-based practice in medicine and nursing as a means of improving healthcare systems. Evidence-based practice has repeatedly been shown to improve patient outcome. Only a small percentage of health care providers implement research findings into practice, due to demanding patient loads, the great amount of journal articles related to their nursing practices and of misunderstandings of the time and procedures implementing practice based on evidence (7).

#### **Significance of study:-**

Nursing is full of completing demands, and the benefits of using evidence based practice are not always apparent. Adoption of an evidence based practice is sometimes misinterpreted as rejection of intuition and experience (8). In a previous study carried out in Saudi Arabia to assess knowledge, attitude and practice of doctors and nurses in primary health care centers revealed poor doctors, nurses knowledge and satisfactory attitude and practice regarding hypertension of pregnancy. (9)

## **2. Subject and Methods**

### **Aim of the study:**

To assess knowledge, attitude and practice of nurses toward evidence based practice. And to find out possible factors influencing their knowledge, attitude and self reported practice.

### **Sample:**

The overall potential number of nurses caring for patients at the time of data collection in all hospital was 300. The returned completely filled questionnaire was 156. Therefore response rate was 52%.

### **Type of the study:**

A descriptive cross sectional study.

### **Setting:**

This study was carried out at Yanbu General hospital located in Yanbu governorate, Madinah Monawarah, kingdom of Saudi Arabia.

### **Study tool:**

Structured self administered questionnaire: Modified evidence-based practice questionnaire for nurses developed according to Upton, D (12), which used to assess knowledge, attitude and practice of nurses regarding evidence based practice. It consists of four main sections:

#### **First:**

Socio-demographic characteristics, educational level, experiences and work related factors.

#### **Second:**

Nurses knowledge: it include enquiring about availability of resources for information, first time of learning nursing research, definition of evidence based practice, some evidence based practice related term as

relative risk, systemic review, odds ratio, publication bias ect., and Barriers to apply evidence based practice in the hospital was assessed (13 items).

#### **\*Scoring system for reply:**

Measuring the score of nurse's knowledge toward evidence based practice.

Sum knowledge was calculated and percentiles was estimated. 25<sup>th</sup> percentile was found to be 13, 50<sup>th</sup> percentile was 15, 75<sup>th</sup> percentile was 21. Three categories was formulated: <25<sup>th</sup> percentile considered poor knowledge, 25 - 75<sup>th</sup> percentile considered around average, >75<sup>th</sup> percentile considered good knowledge.

#### **Third:**

Nurses Attitude towards evidence based practice: it include statements about attitude towards use and benefits of evidence based practice. (8 items).

#### **\*Scoring system:**

Sum attitude was calculated and percentiles was estimated with resultant three categories: 25<sup>th</sup> percentile was 16, 50<sup>th</sup> percentile was 24.75<sup>th</sup> percentile was 30, <25<sup>th</sup> percentile considered negative attitude, 25<sup>th</sup> - 75<sup>th</sup> percentile considered Natural, >75<sup>th</sup> percentile considered positive attitude.

#### **Fourth:**

Nurse's self reported practice of evidence based practice: including formulate a key clinical question, perform literature search efficiently, critically appraise an article dealing with new diagnostic intervention, synthesizing research article, sharing information with peer nurses, integrating evidence into clinical decisions ect. (10 items). and statements about clinical practice guidelines e.g. availability of it in the facility, actively seek for it, use it in practice and able to access it online. (5 items).

\*Scoring system: sum score of nurse's self reported of practice of evidence based practice: Sum self reported practice was calculated and percentiles was estimated. 25<sup>th</sup> percentile was 13, 50<sup>th</sup> percentile was 15, 75<sup>th</sup> percentile was 21. <25<sup>th</sup> percentile considered poor self reported practice, 25<sup>th</sup> - 75<sup>th</sup> considered around average >75<sup>th</sup> considered good self reported practice.

### **Methods:**

1. Approval to carry out the study was obtained by submission of official letters issued from Dean of faculty of Applied Medical Sciences, Taibah University to the director of Yanbu general hospital.
2. Ethical issues was raised by taking verbal consent for participation from every nurse after explaining the aim of the study and confirming confidentially of their data. The researchers emphasized that the participation is voluntary and they have the right to withdraw at any time.

3. A pilot study was conducted for 3day /week for 2weeks and comprised 30Nurses. It aimed at testing applicability of the questionnaire and clarity of its contents and to estimate the time needed to complete it . Time needed to complete the questionnaire was 20 minutes in the average. Modifications were done accordingly and the final form was developed. Data of the pilot study were not included in final results.
4. Over three work shifts, (in Morning shift from 10 Am. - 12 pm. ,in afternoon shift from 4pm. - 6 pm., In night shift from 9-11pm distribution of the questionnaire was carried out three days per week .
5. The data were collected in twelve weeks starting from beginning of March 2012 to the end of May 2012.
6. **Statistical analysis:** Data collected were coded & reviewed prior to computerized entry. Statistical package for social science (SPSS version 18) was used for statistical analysis. Quantitative data were expressed as mean; standard deviation (SD) Qualitative data were expressed as frequency number and percentages .Chi square test was used to analyze cross tables and testing relationships. Significant level was considered at  $p < 0.05$ , and a highly significant level value was considered when  $p < 0.001$ .

#### 7. Operational definitions:-

- 1-**Evidence-based practice:** - is defined as the integration of best research evidence with clinical expertise and patient values and the conscientious, explicit, and judicious use of such evidence in making decisions about the care of individual patients (10).
- 2-**Relative risk:** is the ratio of chance of a disease developing among members of a population exposed to a factor compared with a similar population not exposed to the factor (11).
- 3-**Absolute risk:** The observed or calculated probability of occurrence of an event X, in a population related to exposure to a specific hazard (12) .
- 4- **Odds ratio:** a method of expressing probability (12) .
- 5-**Confidence interval:** a statistical range with a specified probability that a given parameter lies within the range (12).

#### 3. Results:

From Table (1) Study was carried out on 156 nurses their response rate was 52%, participants whose age was below 30 years old were 134 (85.9%), those 30 – 40 years old were 16 (10.3%) and those above 40 years old were 6 (3.8%), They were 145 (92.9%) female nurses and 11(7.1%) males, the majority 120

( 77%) were registered nurses and the majority 103 (66%) were earned diploma of nursing as their highest education as shown in table (1), Most of them 113 (72.4%) work for more than 40 hours per week , the majority 124 (79.1%) have less than 5 years of experience (Table 1). About half of participants (53.8%) reported that they first learn E.B.P in the university only 23.7 % first learn E.B.P. during their practice in hospital , near one thirds of participants reported the process of research evidence ,clinical expertise and integration of patient needs and perspective in making clinical decision as 35.3 , 30.1 , 34.6 in consequence , The resources available to access information were professional journals , Internet in facility and internet outside the facility as reported by 37.8% , 43,6% and 70.5% in consequence , Knowledge of some related terms to E.B.P. revealed highest percentage of clearly known and understandable was for relative risk by 32.1% of participants and the lowest percentage of clearly understand is for heterogeneity as reported by only 10.3% (Table 2). Near fifty percent (48.1%) agree with interested in learning or improving the skills necessary to incorporate E.B.P. Into my practice , also (47.4%) agree with application of E.B.P. is necessary in nursing practice, whereas (72.4%) disagree of feeling disinterested in nursing research , followed by (53.3%) disagree for doesn't take into account patient preferences as shown in table (3). Regarding reported practice of E.B.P. shown in table (4) as highest percentage (13.5%) always done for find best clinical evidence to answer the question , also (13.5%) always evaluating outcome of practice , while highest percentage (61.1%) of sometimes done for critically-appraise an article dealing with new therapeutic mean while highest percent (51.3%) of rarely done for evaluating outcome of practice (Table 4) . Significantly higher number of non Saudian nurses agree regarding the application of E.B.P. is necessary in nursing practice, also significantly higher number of non Saudian agree regarding the literature and research findings are usually in their day to day , whereas non significant difference was observed between non Saudian and Saudian ( $x^2=1.1$ ) as regards feeling disinterested in nursing research (Table 5) .

Table (6) revealed that formulation of key clinical question was always used by (55.4%) of non Saudian nurses versus (44.6%) of Saudian nurses with no statistical significance and rarely used by 42.9% non Saudian versus 57.1% of Saudian , The finding best clinical evidence to answer the question was always by increase higher percent (62.1%) of non Saudian compared to ( 37.9%) literature search was reported as always by 56.6% of non Saudian compared to 43.4% of Saudian and rarely done by 53.3% of non Saudian compared to 46.7% of Saudian with no statistical significant difference , no statistical

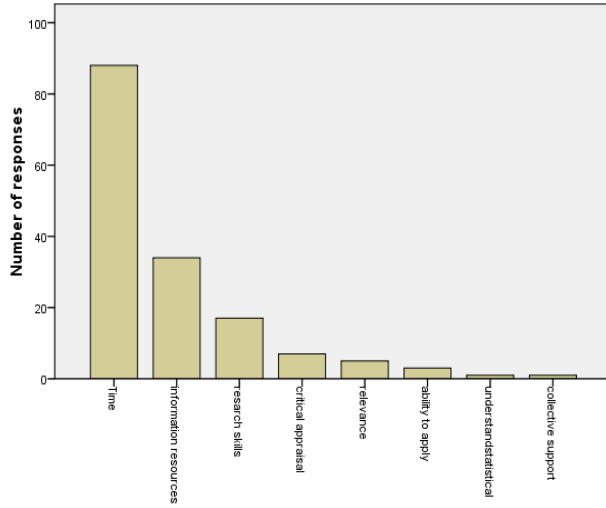
significant difference was observed for increase critically appraising an article dealing with new therapeutics was always carried out as reported by half of non Saudian 50.9% compared to 41.9% of Saudian and rarely done by 53.3% of non Saudian compared to 46.7% of Saudian , Also critically appraising an article dealing with new diagnostic intervention which was always done by 59.3% of non Saudian compared to 40.7% and rarely done by 41.2% of non Saudian as compared to 58.8% of non Saudian as they reported , Non significant difference was also observed for research article where it was reported as always done by 49% of non Saudian versus 51% of Saudian and rarely done by 31.6% of non Saudian versus 68.4% of Saudian , Higher percent of non Saudian report applying E.B.P. to patient care as reported by 60.8% always carried by non Saudian compared to 39.2% non Saudian and rarely done as reported by only 30% of non Saudian versus 70% of Saudian with statistical significance different ( $p<0.05$ ). Also higher percent of non Saudian report integrated evidence into clinical decisions ,sharing information with peer

nurses and evaluating outcome of practice with statistical significance ( $p<0.01$ ). Non significant difference was observed between nurses according to their high educational level neither in knowledge of terms related E.B.P., Attitude toward E.B.P. and practice of E.B.P.

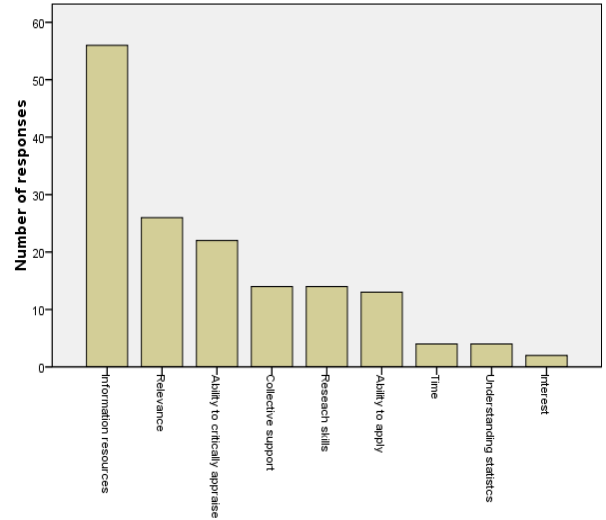
Also non significant difference was shown between Saudian and non Saudian nurses as regard knowledge related E.B.P. Whereas Attitude of Saudian nurses toward E.B.P. shown significantly lower percent than non Saudian whereas ( agree was 37.8% of Saudian and disagree was 68.1% for Saudian , Also practice of E.B.P. shows significantly higher percent of non Saudian always in sum self reported practice 60.5% versus 39.5% of non Saudian , Age group difference for knowledge about E.B.P. related terms shows significantly increase percent of knowledge with age with increase trend with age. Attitude towards E.B.P. significantly increase with age although self reported practice increase with age yet no significant difference observed (Table 7.)

Table (1) General characteristics of participant nurses

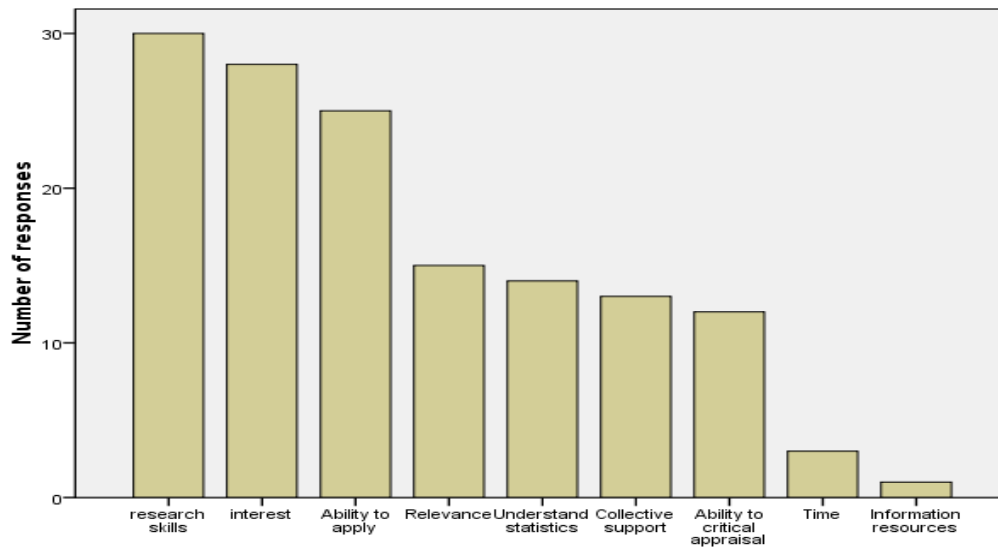
Parameters	Number	%
Age :		
< 30 year	134	85.9
30 - 40 year	16	10.3
> 40 year	6	3.8
Gender :		
Male	11	7.1
Female	145	92.9
Nationality :		
Saudian	78	50
Others	78	50
Position :		
Director of nursing	2	1.2
Nurse manage	6	3.8
Charge nurse	14	9
Head nurse	14	9
Register nurse	120	78
Highest educational degree earned :		
Diploma	103	66
B. S. N.	52	33.3
Master	1	0.6
Years of experience in current practice area:		
< 5 years	124	79.1
5 - 10 years	28	17.9
> 10 years	4	3
Had Saudian commission for health specialist license		
No	34	21.8
Yes	122	78.2
Work hours per week :		
20 - 30 hour	1	0.6
31 - 40 hour	42	26.9
> 40 hour	113	72.4



**Figure (1) First ranked main barrier against evidence based practice**



**Figure (2) Secondly ranked main barrier against evidence based practice**



**Figure (3) Thirdly ranked main barrier against evidence based practice**

**Table (2): Assessment of nurses' knowledge related to evidence based practice:-**

Assessment of nurses' knowledge about evidences based practice:-	No	%				
1-First learn nursing research :						
No where	32	20.2				
In the university	84	53.8				
During practice	37	23.7				
Others	3	1.9				
2-The process of E.B.P. in making clinical decisions includes :						
research evidence	55	35.3				
clinical expertise	47	30.1				
integrate patient needs &perspectives	54	34.6				
3-Availability of resources to access information through :						
professional journals	59	37.8				
internet in facility	68	43.6				
internet outside the facility	110	70.5				
4-knowledge of some terms related to E.B.P						
	Level of Understanding					
	Clearly understood		somewhat		not understood	
	No	%	No	%	No	%
Relative risk	50	32.1	56	35.9	50	32.1
Absolute risk	40	25.6	61	39.1	55	35.1
Odds	23	14.7	55	35.3	78	50
Meta analysis	24	15.4	48	30.8	84	53.8
Confidence interval	28	17.9	47	30.1	81	51.9
Heterogeneity	16	10.3	54	34.6	86	55.1
Publication bias	26	16.7	53	34	77	49.4

**Table (3):- Attitude of nurses towards use & benefits of Evidence Based Practice**

Attitude of nurses towards use & benefits of Evidence Based Practice :-	Disagree	Neutral	Agree
1-Application of EBP is necessary in nursing practice	35.3%	17.3%	47.4%
2-Literature and research findings are usually in my day to day practice	39.7%	25%	35.3%
3-I need to increase the use of evidence in my daily practice	38.4%	19.3%	42.3%
4- Interested in learning or improving the skills necessary to incorporate EBP into my practice.	33.4%	18.5%	48.1%
5-E.B.P. Improves the quality of patient care.	35.9%	19.2%	44.9%
6-Helps me make decisions about patient care.	40.3%	16.7%	43%
7-Doesn't take into account patient preferences.	53.3%	28.8%	17.9%
8- I feel disinterested in nursing research.	72.4%	17.3%	10.3%

**Table(4): Self reported practice of Evidence Based Practice among studied nurses**

Self reported practice items	Always		Sometimes		Rarely	
	No	%	No	%	No	%
1-Formulate a key clinical question	14	9	77	49.4	65	41.7
2-Find best clinical evidence to answer the question	21	13.5	69	44.2	66	42.4
3-Perform literature search efficiently	15	9.6	88	56.4	53	34
4-Critically appraise an article dealing with new therapeutic	15	9.6	86	61.1	55	35.3
5-Critically appraise an article dealing with new diagnostic intervention	17	10.9	85	54.5	54	34.6
6-Synthesizing research article	19	12.2	88	56.5	49	31.4
7-Apply EBP to patient care	10	6.4	72	46.2	74	47.4
8-Integrating evidence into clinical decisions	13	8.3	68	43.6	75	48
9-Share information with my peer nurses	17	10.9	68	43.6	71	45.5
10-Evaluating outcome of practice	21	13.5	55	35.2	80	51.3

Table (5): Attitude towards Evidence Based Practice among nurses according to their nationality

Attitude towards use and benefits of EBP	Saudian		Non saudian		Significance
	No	%	No	%	
1-Application of EBP is necessary in nursing practice: Disagree Neutral agree	38 13 27	69.1 48.1 36.5	17 14 47	30.9 51.9 63.5	X2=13.4 p<0.001 H.S.
2-Literature and research findings are usually in day to day practice Disagree Neutral Agree	42 15 21	67.7 38.5 38.2	20 24 34	32.3 61.5 61.8	X2=12.9 p<0.05 significant
3-I need to increase the use of evidence in daily practice Disagree Neutral Agree	44 14 20	73.3 46.7 30.3	16 16 46	26.7 53.3 69.7	X2=23.4 p<0.001 H.S.
4- Interested in learning or improving the skills necessary to incorporate EBP into my practice. Disagree Neutral Agree	37 14 27	71.2 48.3 36	15 15 48	28.8 51.7 64	X2=15 p<0.001 H.S.
5-E.B.P. Improves the quality of patient care. Disagree Neutral agree	37 14 27	66.1 46.7 38.6	19 16 43	33.9 53.3 61.4	X2=9.57 p<0.05 significant
6-EBP helps in making decisions about patient care. Disagree Neutral agree	43 14 21	68.3 53.8 31.3	20 12 46	31.7 46.2 68.7	X2=17.8 p<0.001 H.S.
7-EBP doesn't take into account patient preferences. Disagree Neutral agree	38 25 15	45.8 55.6 53.6	45 20 13	54.2 44.4 46.4	X2=1.2 p>0.05 Insignificance
8- I feel disinterested in nursing research. Disagree Neutral Agree	55 13 10	48.7 48.1 62.5	58 14 6	51.3 51.9 37.5	X2=1.1 P>0.05 Insignificance

Table (6): practice of Evidence Based Practice among saudian and non saudian nurses :

Variables	Saudian		Non saudian		X2
	No	%	No	%	
1-Formulate a key clinical question Always Sometimes Rarely	8 41 29	57.1 53.2 44.6	6 36 36	42.9 46.8 55.4	X2=1.36 P>0.05 N . S.
2-Find best clinical evidence to answer the question Always Sometimes Rarely	13 40 25	61.9 58 37.9	8 29 41	38.1 42 62.1	X2=6.8 P<0.05 Significant
3-Perform literature search efficiently Always Sometimes Rarely	7 48 23	46.7 54.5 43.4	8 40 30	53.3 45.5 56.6	X2=1.7 p>0.05 N. S.
4-Critically appraise an article dealing with new therapeutic Always Sometimes Rarely	7 44 27	46.7 51.2 49.1	8 42 28	53.3 48.8 50.9	X2=0.13 p>0.05 N. S.
5-Critically appraise an article dealing with new diagnostic intervention Always Sometimes Rarely	10 46 22	58.8 54.1 40.7	7 39 32	41.2 45.9 59.3	X2=2.9 p>0.05 N. S.
6-Synthesizing research article Always Sometimes Rarely	13 40 25	68.4 45.5 51	6 48 24	31.6 54.5 49	X2=3.3 p>0.05 N. S.
7-Apply EBP to patient care Always Sometimes Rarely	7 42 29	70 58.3 39.2	3 30 45	30 41 60.8	X2=7 P<0.05 Significant
8-Integrating evidence into clinical decisions Always Sometimes Rarely	10 41 27	76.9 60.3 36	3 27 48	23.1 39.7 64	X2=12.5 P<0.01 Significant
9-Share information with my peer nurses Always Sometimes Rarely	12 45 21	70.6 66.2 29.6	5 23 50	29.4 33.8 70.4	X2=21.8 p<0.001 H.S
10-Evaluating outcome of practice Always Sometimes Rarely	12 38 28	57.1 69.1 35	9 17 52	42.9 30.9 65	X2=15.6 p<0.001 H.S



Table (7) :- Factors affecting knowledge, attitude and practice of evidence based practice among nurses

Variables	Knowledge about EBP						Significance
	Poor		Around average		Good		
Level of education:- Diploma B.C. Master	25 15 00	62.5 37.5 00	45 23 1	65.2 33.3 1.4	33 14 00	70.2 29.8 00	X <sup>2</sup> = 0.58 p >0.05 N. S.
Nationality : Saudian Non saudian	19 21 52.5	47.5	34 35	49.3 50.7	25 22	53.2 46.8	X <sup>2</sup> =0.3 p >0.05 N. S.
Age : < 30 year 30 - 40 year > 40 year	40 00 00	100 00 00	54 13 2	78.3 18.8 2.9	40 3 4	85.1 6.4 8.5	X <sup>2</sup> =15.5 p<0.05 Significance
Level of education:- Diploma B.C. Master	Attitude towards E. B. P						Significance
	Negative		Neutral		Positive		
	33 14 00	70 29.8 00	42 22 00	65.6 34.4 00	28 16 1	62.2 35.6 2.2	X <sup>2</sup> =0.47 p >0.05 N. S.
Nationality : Saudian Non saudian	32 15	68.1 31.9	29 35	45.3 54.7	17 28	37.8 62.2	X <sup>2</sup> =9.4 p<0.05 Significance
Age : < 30 year 30 - 40 year > 40 year	44 3 00	93.6 6.4 00	52 6 6	81.2 9.4 9.4	38 7 00	84.4 15.6 00	X <sup>2</sup> =9.4 p<0.05 Significance
Level of education:- Diploma B.C. Master	Self reported practice of evidence based practice						Significance
	Good		Around average		Poor		
	30 9 00	76.9 23.1 00	48 26 00	64.9 35.1 00	25 17 1	58.1 39.5 2.3	X <sup>2</sup> =2.9 p >0.05 N. S.
Nationality : Saudian Non saudian	28 11	71.8 28.2	33 41	44.6 55.4	17 26	39.5 60.5	X <sup>2</sup> =9.4 p<0.05 Significance
Age : < 30 year 30 - 40 year > 40 year	36 3 00	92.3 7.7 00	65 5 4	87.8 6.8 5.4	33 8 2	76.7 18.6 4.7	X <sup>2</sup> =6.7 p >0.05 N. S

#### 4. Discussion

In most developed countries, use of EBP is the goal of public services (6). In the past two decades, there has been a more conscientious attempt to use EBP in various health settings. (14).The degree to which EBP is used varies among practitioners and across practice settings. Thyer(15) noted that many federal agencies have emphasized the use of evidence-based interventions and are now linking their grants to translational research.

Although the utilization of research in nursing practice has increased, there are differences in the nurses' education level regarding research utilization. Moreover research was highly dependent on the culture of the hospital, and meaning the provision of resources (16).

Evidenced based nursing practice is an approach that enables nurses to provide the highest quality care based on the best evidence that is available, which in

turn, positively affects the outcome of nursing interventions. To improve patient outcomes currently and in the future, it is important that an evidence-based approach to nursing care be incorporated into clinical practice settings. Findings of the present study may contribute to the existing body of knowledge about evidence – based practice and add essential information about knowledge, practice , and attitude of nurses related to evidence-based practice.

This study included (156) respondents who returned complete, correct and valid questionnaires.

On assessing EBP two dimensions should be discussed, one related to the facility system and availability of information resources, and the second is concerned with individual knowledge, skills and practice.

Current study showed lower than expected percent of those first learn EBP during their nursing

academic study program, it was only 53.8%, while 23.7% first learn EBP during their work practice in the hospital and 20% didn't learn about EBP anywhere.

In this respect, previous studies (17-19) who evaluated nurses' knowledge and skills to critically appraise related research, found low scores of participants. They highlighted defective areas such as the need for additional learning as increased knowledge about available evidence, the skills to search for and critique research, the ability to read, interpret, and translate research into practice, and the skills to evaluate the strength of the evidence. Previous studies (20,21) who reported that many nurses are struggling or lack the knowledge and skills to implement evidence-based care. Numerous reasons for the failure to implement evidence-based practice (EBP) have been cited, including: (a) lack of knowledge regarding evidence-based strategies, (b) misperceptions of or negative attitudes about research and evidence-based care, (c) lack of knowledge regarding how to search for and appraise evidence, (d) demanding patient workloads, (e) organizational constraints (e.g., lack of administrative support or incentives), (f) patient expectations (e.g., parents who demand antibiotics for their child's upper respiratory infection when they are not indicated), (g) fears about practicing differently than peers, and (h) overwhelming amounts of information in medical and nursing journals as well as textbooks.

Main reported barriers against implementing EBP in the current study were lack of time, lack of information resources and poor research skills. All of which can be improved through the health facility. Without the support of hospital and administrators, which supply and encourage nurses to access current best evidence, it will take a concerted effort to fully use evidence-based nursing within clinical practice settings.

Current study shows that nurses' knowledge, attitude, and practice increase with age regarding evidence-based practice, statistically significant increase percentage of knowledge, and attitude among older nurses. The finding in this study is congruent with Koehn study (22) who surveyed general practice nurses in Australia to assess their knowledge and relationship to EBP and found that younger nurses rated their information technology skills higher than did older nurses. McEwen (23) surveyed nurses of various ages, experience, and level of education at a large hospital and found moderate scores on practice and attitude towards EBP, with lower scores on skills. In the current study, no significant difference was observed between nurses according to their levels of education, neither in knowledge of terms related to evidence-based practice, attitude or self-reported practice of EBP.

Although the utilization of research in nursing practice has increased, there are differences in the nurses' education level regarding research utilization. Moreover, research was highly dependent on the culture of the hospital, meaning the provision of resources and the support that nurses had (16).

In this respect, Koehn pointed out that many nursing scholars believe evidence-based nursing practice will fill the gaps that are present between research, theory, and practice. Unfortunately, many nurses in clinical practice do not understand the concept of evidence-based nursing or how to incorporate this approach into general clinical practice settings.

The results of this study revealed a positive attitude of participant nurses towards improving the skills necessary to incorporate EBP. This explanation is in line with the findings reported by Melnyk (20), which indicated that a positive attitude towards research utilization could enhance participation in EBP-related activities. Nationality shows a difference in this study, where a significant higher number of non-Saudian agree with application of EBP compared with Saudi, where they represent different schools of graduation.

No significant difference was observed between non-Saudi and Saudi towards their feeling disinterested in nursing research. Emphasis should be placed on the positive findings of this study that nurses are ready for change and willing and interested to implement EBP. In particular, insights gained from this study might help health care institutions support nurses to apply EBP in their practice.

### Conclusion

The observed lack of the required knowledge and practice of evidence-based practice among nurses in the current study raise the issue of applying evidence-based practice in hospitals and other health care facilities and assessing that during reviewing the health institution for renewal of license or accreditation.

### Recommendation:

In the light of the present study findings, the following suggestions are recommended:

The area of this research is needed with larger sample size and different methodologies to confirm the present results.

Descriptive research can provide a baseline assessment for strategic planning efforts to move organizations toward evidence-based practice and identify the best strategies for implementing EBP.

Furthermore, education about research, either as part of typical nursing education or through continuous education and workshop for nursing staff is of great importance.

Administrators can support the development of EBP by allowing nurses time to learn skills related to EBP, such as searching bibliographic databases or learning how to critically evaluate research studies

- 1- To add and inforce teaching E.B.P. in university courses for health science .
- 2- To incorporated E.B.P. in hospital policy and plans to improve care provided incorporated

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#### References

1. Schubert .F., S.M., PhD, Intan. A., Xue. Z., Brendan .L., Yun. C., and Yin.T., 2011. Nurses' Perception of Evidence-Based Practice at the National University Hospital of Singapore. *The Journal of Continuing Education in Nursing.* 42(11.)40
2. McNeece, C.A., & Thyer, B. A., 2004. Evidence-based practice and social work. *Journal of Evidence-Based Social Work.*, 1: 7-25.
3. Levin, R., Feldman, H., 2006. *Teaching Evidence-based Practice in Nursing. A Guide for Academic and Clinical Settings.*: New York: Springer.
4. Greiner A.C. & Knebel, E., 2003. *Health professions education. a bridge to quality.* National Academies.: Washington DC.
5. Oranta, O., Routasalo, P., Hupli, M., 2002. Barriers to and facilitators of research utilization among Finnish registered nurses. *Journal of Clinical Nursing*, 11: 205-213.
6. Joint Commission on Accreditation of Healthcare Organizations,2008. Retrieved, Performance Measurement. World Wide Web February 3 [cited; <http://www.jointcommission.org/>].
7. Ritter , B., 2001.Considering evidence based practice nurse practioner. *Proquest Central*, 26(5): p. 63.
8. Mohammad B.S. Gandeh ,W.A., 2010.Milaat Saudi society of family and community medicine .evidence based nursing (5) 38-40
9. Nutley, S., Walter, I., & Davies, H. T. , 2009. Promoting evidence-based practice: Models and mechanisms from cross-sector review. *Research on Social Work Practice.*, 19: 552-559.
- 10.Regehr, C., Stern, S., & Shlonsky, A. , 2007. Operationalizing evidence-based practice: The development of an institute for evidence-based social work. *Research on Social Work Practice.*, 17: 409-416.  
<http://medical-dictionary.thefreedictionary.com/>
11. Upton, D., & Upton, P., 2006. Development of an evidence-based practice questionnaire for nurses. *Journal of Advanced Nursing*, 454-458(54).
12. Fixsen, D.L., Blase, K. A., Naoom, S. F., & Wallace, 2009. Core implementation components. *Research on Social Work Practice.*, 19: 531-540.
13. Rodgers, S.E., 2000. A study of the utilization of research in practice and the influence of education 2000. *Nurse Education Today.*: Harcourt Publishers Ltd
14. Thyer, B.A., 2008.The quest for evidence-based practice?We are all positivists! *Research on Social Work Practice*, 18: 339-345.
15. Ervin, N.E., 2002. Evidence-based nursing practice: Are we there yet? . *The Journal of the New York State Nurses' Association.*, 33(2): 11-16.
16. McCaughan, D., Thompson, C., Cullum, N., Sheldon, T. A., & Thompson, 2002. Acute care nurses' perceptions of barriers to using research information in clinical decision-making. *Journal of Advanced Nursing.*, 39: 46-60
17. Paramonczyk, A., 2005. Barriers to implementing research in clinical practice. *Canadian Nurse.*, 101(3) 12-15.
18. Mills, J., Field, J., & Cant, R., 2009. The place of knowledge and evidence in the context of Australian general practice nursing. *Worldviews on Evidence-Based Nursing.*, 6: 219-228.
19. Melnyk, B., Fineout-Overholt, E., Stone, P.& Ackerman, M. , 2000. Evidence-based practice: the past, the present, and recommendations for the millennium. *Pediatr Nurs.* 26.: 77-80.
20. McKenna HP, A.S., Keeney S. , 2004. Barriers to evidence-based practice in primary care. *J Adv Nurs.*, 45(2) 178-89.
21. Koehn ML, L.K., 2008. Nurses' perceptions of evidence-based nursing practice *J Adv Nurs.*, 62(2): 15.
22. McEwen, M., 2002. Application of theory in nursing practice. *Theoretical basis for nursing.*, M. McEwen & E. M. Wills (Eds.): Philadelphia: Lippincott, Williams & Wilkins.

## Early detection of Alzheimer's disease using structural MRI: A research idea

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**Abstract:** Alzheimer's disease (AD) is a common progressive neurodegenerative disorder that is not currently diagnosed until a patient reaches the stage of dementia. There is an urgent need to identify AD at an earlier stage, so that treatment can begin early. Structural imaging based on magnetic resonance imaging (MRI) is an integral component of the clinical assessment of patients with suspected AD. Rates of brain atrophy could be assessed in specific regions such as the hippocampus, entorhinal cortex, temporal and parietal lobes, and ventricles. Structural brain MRI is becoming increasingly used in the early diagnostics of AD. Volumetry and pattern recognition techniques for measuring cortical thinning and automated classification approaches that assess the overall pattern of atrophy seem to show promise for the early diagnosis of AD. The study is aimed at developing new pattern recognition techniques and automatic classifiers to reliably detect AD in its early stages. Data used in the preparation of this proposal is supposed to be obtained from the Alzheimer's disease neuroimaging initiative (ADNI) database. Study will begin with pre-processing of MRI images which includes correction of inhomogeneities, de-noising, registration to the stereotaxic space e.g., using a linear transform and cross normalization of the MRI intensity followed by data modulation. Brain tissue will be segmented into white matter (WM), grey matter (GM) and cerebrospinal fluid (CSF) by the SMP software. Customized tissue probability maps (TMPs) have to be created for bias correction. For feature reduction and feature selection, datasets will be inserted into a linear support vector machine (SVM). After training a model by a sub-group, cross-validation by another sub-group will be used to achieve SVM parameter optimization. We also try to develop a better classifier e.g. Neural Network for automate classification. It is expected that using structural MRI to predict AD during early stages will allow for diagnosis and treatment before irreversible neurodegeneration and functional impairment have occurred. The aim is to improve the classification accuracy that can be achieved by combining features from different structural MRI analysis techniques.

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### 1. Introduction

#### 1.1. Definition

Alzheimer's disease (AD) is a neurodegenerative disease of the brain that causes changes in brain functions. AD usually affects people over the age of 65 years, resulting in a progressive decline in memory as well as, thinking, language and learning capacity. Age is the strongest predictor for the development and progression of AD; with the rapidly aging population of our society, AD clearly poses a major health problem (1). The pathophysiology of AD is related to the injury and death of neurons, especially in those areas of the brain involved with memory and learning. AD is the most common form of dementia, accounting for 50% to 75% of all dementia cases, with a greater proportion among older populations. AD should be differentiated from normal age-related declines in cognitive function, which are more gradual and associated with less disability. AD often starts with mild symptoms and ends with severe brain damage. People with dementia lose their abilities at different rates. On average, AD

patients live from 8 to 10 years after being diagnosed, although the disease can last for as many as 20 years (1, 2). Mild cognitive impairment (MCI) is a transitional state between normal aging and dementia. MCI is associated with an increased risk for dementia. Such patients are able to live independently, are aware of their memory changes, and typically show problems with delayed recall, although non memory cognitive domains can also be impaired (3, 4).

#### 1.2. Epidemiology of AD

Advances in medical technology have helped increase life expectancy, but age-associated cognitive impairment often diminishes the quality of life for the increasing numbers of older adults. AD is currently the most common form of dementia. . In 2005, an estimated 24 million people around the world suffered from dementia. According to the 2010 World Alzheimer report, an estimated 35.6 million people worldwide are living with dementia at a total cost of more than US\$600 billion in 2010, and the

incidence of AD throughout the world is expected to double in the next 20 years (5, 6). By 2040, it is predicted that more than 81 million people worldwide will suffer from dementia. Deaths, because of AD have been rising dramatically while other major causes of death have been on the decline. AD is the sixth leading cause of all deaths in the United States and the fifth leading cause of death in Americans aged 65 years and older. Between 2000 and 2008, deaths due to AD increased by 66% whereas heart disease deaths decreased by 13%, stroke deaths by 20%, and prostate cancer-related deaths by 8% (5, 6).

AD inflicts a terrible toll on patients, their families, and society in general. Most experts agree that treatment is most beneficial if applied early, before significant, potentially irreversible neurodegeneration and functional impairment has occurred (4, 5). Every 69 seconds, one person in America develops AD; by 2050, the time is expected to accelerate to every 33 seconds. Over the coming decades, the baby boom population is projected to add 10 million people to these numbers. By 2050, the incidence of AD is expected to approach nearly a million people per year, with a total estimated prevalence of 11 to 16 million people. Dramatic increases in the numbers of “oldest-old” (those aged 85 years and older) across all racial and ethnic groups will also significantly affect the numbers of people living with AD while the number of Americans aged 65 and over with AD is projected to reach 13.2 million in 2050 compared with 4.5 millions in 2000. If present trends continue, the cost of caring for the expected increase in the number of AD patients will bankrupt public healthcare systems (4, 5).

An estimated 5.4 million Americans have AD; approximately 200,000 65-year-olds with AD comprise the younger onset of AD population. In 2010, nearly 15 million family and other unpaid caregivers provided an estimated 17 billion hours of care to people with AD and other dementias— a contribution valued at more than \$202 billion (4-6). Medicare payments for services to 65+-year-old beneficiaries with AD and other dementias are almost 3 times higher than for beneficiaries without these conditions. Total payments in 2011 for healthcare, long term care, and hospice services for 65+-year-olds with AD and other dementias were estimated to be \$183 billion (not including the contributions of unpaid caregivers) (4-6).

### 1.3. Diagnosis

#### 1.3.1. Symptoms and signs

AD can affect different people in different ways, but the most common symptom pattern begins with gradually worsening difficulty in remembering new information as the disruption of brain cell

function usually begins in regions involved in forming new memories. As damage spreads, individuals experience other difficulties. The following are warning signs and symptoms of AD (3-5):

- Memory loss that disrupts daily life
- Challenges in planning or solving problems
- Difficulty completing familiar tasks at home, work, or leisure
- Confusion with time or place
- Trouble understanding visual images and spatial relationships
- New problems with words in speaking or writing
- Misplacing things and losing the ability to retrace steps
- Decreased or poor judgment
- Withdrawal from work or social activities
- Changes in mood and personality

#### 1.3.2. Laboratory findings

Lab tests may be done to either categorize dementia or negatively exclude other possible causes of a person’s symptoms, such as levels of certain minerals or chemicals in the blood being too high or too low, liver disease, abnormal thyroid levels, or nutritional problems, such as folate or vitamin B12 deficiencies. Some of the most common laboratory tests include the following:

- Complete Blood Count (CBC)
- Serum electrolytes
- Thyroid panel
- Vitamin B12
- Neurosyphilis serology
- ELISA
- Western Blot
- Urine toxicology
- ESR
- EEG

#### 1.3.3. Imaging findings

The methods used for early detection of AD include clinical tests, as well as computerized tomography (CT), magnetic resonance imaging (MRI), single photon emission computed tomography (SPECT), positron emission tomography (PET), and cerebrospinal fluid (CSF) biomarkers (5-7). Traditionally, neuroimaging techniques have been categorized as either structural or functional, according to the primary information they provide. However, methods generally used to look at structure can also be altered to observe function (e.g., functional MRI). Similarly, traditional functional methods, such as PET, can also be used to view structure (e.g., amyloid plaque imaging). Commonly used structural methods include CT and MRI. Studies

of brain function are often done using SPECT, with technetium to measure blood flow, or PET, with the 2-deoxy-2-[<sup>18</sup>F] fluoro-D-glucose (FDG) tracer to measure glucose metabolism. In clinical practice, imaging studies are usually used to increase diagnostic accuracy to assist with treatment planning. The most recent American Academy of Neurology Practice Parameter guidelines recommend structural neuroimaging with either a non contrast CT or MRI scan in the initial assessment of patients with dementia (7-9)

The advent of computer-based methods for quantitative MRI has allowed for efficient quantification of AD-related atrophy across the brain. MRI offers good contrast between the different soft tissues of the body and provides high-resolution (~1 mm) information, making it especially useful in imaging the brain structure. Gadolinium contrast agents are sometimes used to enhance the visualization of brain lesions. In clinical practice, structural MRI scanning is widely used, and radiologists interpret results based on visual readings (10, 11). However, image analysis programs that quantify regional volumes in MRI have shown that medial temporal or hippocampal atrophy measures can distinguish patients with a clinical diagnosis of AD from controls. Although hippocampal atrophy can predict memory progression, these changes might not be specific to AD and might occur in other dementia disorders (7, 10, and 12).

Current consensus statements have emphasized the need for early recognition and the fact that a diagnosis of AD can be made with high accuracy by using clinical, neuropsychologic, and imaging assessments. Magnetic resonance (MR) or CT imaging is recommended for the routine evaluation of AD. Coronal MR images can be useful for documenting or quantifying atrophy of the hippocampus and entorhinal cortex, both of which occur early in the disease process. Both volumetric and subtraction MR techniques can be used to quantify and monitor dementia progression and rates of regional atrophy. MR measures are also increasingly being used to monitor treatment effects in clinical trials of cognitive enhancers and antidementia agents.

PET and single photon emission CT offer value in the differential diagnosis of AD from other cortical and subcortical dementias and may also offer prognostic value. In addition, PET studies have demonstrated that subtle abnormalities may be apparent in the prodromal stages of AD and in subjects who carry susceptibility genes. PET ligands are in late-stage development for the demonstration of amyloid plaques, and human studies have already

begun. Functional MR-based memory challenge tests are in development as well.

The principal agents used in brain SPECT of patients with AD are <sup>99m</sup>Tc hexamethyl propyleneamine oxime (<sup>99m</sup>Tc HMPAO) and <sup>99m</sup>Tc ethylcysteinate dimer (<sup>99m</sup>Tc ECD). These agents accurately measure cerebral blood flow and making them valuable in identifying the reduced cerebral blood flow in the temporoparietal region seen in patients with AD (13).

On a perfusion MRI using dynamic susceptibility contrast-enhanced MRI— a method utilizing rapid T2\*-weighted imaging of the brain during intravenous injection of a bolus of paramagnetic contrast material— reduced relative cerebral blood volume can be seen in the temporal and parietal regions of patients with AD, which correlates with the cerebral blood flow reduction seen on PET and SPECT. Unlike PET perfusion imaging, MR perfusion imaging takes less time to perform, involves no radiation exposure, and is readily available on any MR unit capable of echo-planar imaging (14).

Diffusion-weighted MRI detects alterations in microscopic water motion within tissues, which is usually measured by the apparent diffusion coefficient value. An increased rate of water diffusion has been reported in the hippocampal gyri of AD patients, likely caused by the disruption of membranes and myelin sheaths combined with the fragmentation of axons and dendrites or by the expansion of extracellular fluid from the inflammatory response (15).

MR brain activation studies that assess the degree of brain activation during memory tasks using a blood oxygenation level-dependent technique offer another promising method for studying AD. Studies of AD patients have shown decreased activation of the left medial temporal lobe during auditory memory tasks and decreased activation of the right parietal region in response to visual memory tasks. Functional MR imaging has proved to be a powerful research technique to aid in identifying regions of the brain activated by particular stimuli and tasks. With this technique, regional brain activity is measured according to local changes in deoxyhemoglobin concentration in response to various stimuli and tasks. In brief, rapid T2\*-sensitive imaging, usually gradient-echo echo-planar imaging, is performed during presentation of a stimulus or performance of a specific task and during rest periods. A voxel-by-voxel statistical comparison is then performed with images obtained during the stimulus/task periods versus those obtained during the rest periods, creating a statistical-activation map that can be “thresholded”

and presented as a color overlay on anatomic T1-weighted images (15, 18).

MR spectroscopy is a technique that can monitor the metabolic changes that accompany brain structural changes and therefore would be expected to play a valuable role in the imaging of AD. The presence of senile plaques is associated with neuronal loss that is reflected in a decrease in the neuronal marker N-acetylaspartate. This N-acetylaspartate decrease can be readily detected on MR spectroscopy (16).

#### 1.3.4. Diagnostic criteria

Clinical criteria provide sensitivity of greater than 90% for diagnosing dementia of any type, including AD, in specialized clinical settings such as memory disorders clinics; however they have a specificity of less than 70% for the actual diagnosis of AD (16).

The clinical standards used to diagnose AD were first defined in 1984. These standards require insidious onset; gradual progression of memory deficits; a focus on early deficits of recent memory; and later impairment of orientation, judgment, problem solving, community and home living, and personal care. In addition to a diagnosis of clinically define AD, the diagnoses of probable AD and possible AD can be rendered; both of these diagnoses allow for variations in onset, presentation, and course. The term “probable Alzheimer's disease” refers to memory deficits evident in neuropsychological testing (Mini-Mental State Examination [MMSE] score  $\leq$  23) and progressive worsening of memory and deficits in two or more cognitive functions, as documented by clinical and neuropsychologic testing.

The cognitive functions discussed thus far are measured by a battery of clinical and psychometric tests, such as the MMSE and clinical dementia rating. Normal cognitive performance scores for the MMSE are greater than or equal to 27.6 (maximum score = 30); for the clinical dementia rating, they are less than 0.5 on a scale of 0–3.

#### 1.4. Study Background

The goal of early MRI studies in AD has been to identify general evidence for brain damage that was specifically associated with AD and with the severity of the clinical symptoms (5, 17). Advances in medical imaging systems have provided a wide spectrum of valuable and complementary information about a patient's pathology, anatomy, and physiology. Information produced by CT, MRI, SPECT, PET, and CSF differs in dimensionality, scale, extent, and biological origin. For example, structural MRI has been successful in the early

detection of the effects of AD on the brain even in earlier stages of the disease when clinical symptoms are not fully expressed and the regional brain damage may be limited. Thus, MRI measurements, primarily in the gray matter (GM), could be sensitive markers of the disease and assist early diagnosis (6, 18).

Volumetric changes to brain structure could be assessed by MRI of specific regions such as the hippocampus, entorhinal cortex, temporal and parietal lobes, and ventricles [12-13]. Current clinical MRI scanners with 1.5T or 3T magnets allow for the acquisition of high resolution digital images of the brain in exquisite structural detail, with excellent tissue contrast and spatial resolution of  $\leq$ 1 mm (4).

#### 1.5. Problem Statement

AD is a common progressive neurodegenerative disorder that is not currently diagnosed until a patient reaches the stage of dementia. A pressing need exists to identify AD at an earlier stage, so that treatment, when available, can begin early (7). According to the World Alzheimer Report 2011, most people living with dementia have not received a formal diagnosis. In high-income countries, only 20-50% of dementia cases have been recognized and documented in primary care. This treatment gap is certainly much greater in low- and middle-income countries, with one study in India suggesting that 90% of such cases remain unidentified. If these statistics are extrapolated to other countries worldwide, it suggests that approximately 28 million of the 36 million people with dementia have not received a diagnosis, and therefore do not have access to treatment, care, and organized support that getting a formal diagnosis can provide.

This is clearly a major concern, given that the world's population is growing older and new cases of dementia and AD are increasing relentlessly; as a result, earlier diagnosis and early intervention are important mechanisms by which the treatment gap can be closed. With increasing life expectancy across the world, the number of elderly people at risk of developing dementia is growing rapidly. The prevalence of dementia rises steeply with age, doubling every 4–5 years from the age of 60, meaning that more than one third of individuals over 80 years of age are likely to develop dementia. AD remains the most common cause of dementia in all age groups (19).

#### 1.6. Study Significance

Pathological processes and brain abnormality in AD can be detected using structural MRI, which is becoming increasingly popular in the early diagnostics of AD (3). Quantitative structural

MRI is sensitive to the neurodegeneration that occurs in mild and preclinical AD, and is predictive of decline to dementia in individuals with mild cognitive impairment. Objective evidence of ongoing brain atrophy is critical for risk/benefit decisions once potentially aggressive, disease-modifying treatments become available. Recent advances have paved the way for the use of quantitative structural MRI in clinical practice, and initial clinical use has been promising (10, 12). Early detection and diagnosis of cognitive impairment confers that early detection and prevention (4, 17, and 20):

- Leads to minimum cognitive decline;
- Helps facilitate treatment or management of coexisting medical conditions that worsen cognitive function;
- Predict of conversion to AD;
- Allows prompt evaluation and treatment of reversible or treatable causes of cognitive impairment;
- Allows potential management of symptoms with medication or other interventions;
- Aids in the management of possible behavioral symptoms.

## 1.7. Objectives

### 1.7.1. General objectives

The general objective of the study is to find a method for early detection of AD using structural MRI. In this proposal, “early diagnosis” refers to one’s ability to diagnose AD at a very early stage, before symptoms and clinical signs have reached the stage at which a diagnosis of clinically probable AD can be made according to currently recommended criteria.

### 1.7.2. Specific objectives

The specific objectives of the proposed study are:

- To improve the latest techniques used in structural MRI for measuring the progression of MCI and early AD;
- To develop methods of extracting quantitative and semi-quantitative data from structural MRI to be applied in early diagnosis of AD;
- To outline new means for feature extraction and feature selection;
- To determine an automated technique for extracting AD-specific data from cross-sectional and serial structural MRI;
- To improve methods for sMRI-based volumetric assessment in ROI;
- To implement a new and more precise classifier to clarify with greater accuracy the

transitional zone between the healthy aging and the first manifestations of AD; and

- To reinforce the place of structural MRI as a powerful biomarker of the stage and intensity of AD.

## 2. Proposed Methodology

### 2.1. Research Design

The proposed research is a diagnostic image processing study based on data acquired from structural MRI neuroimaging. It is aimed at developing new pattern recognition techniques to reliably detect AD in its early stages (Fig. 1).

### 2.2. Research Method

#### 2.2.1. Materials

Data used in the preparation of this proposal were obtained from the Alzheimer’s ADNI database (<http://www.loni.ucla.edu/ADNI>). The ADNI is a multi-center study assessing neuroimaging for diagnosis and longitudinal monitoring. The ADNI was launched in 2003, and its primary goal has been to test whether serial MRI, PET, other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of MCI and early AD. The determination of sensitive and specific markers of very early AD progression is intended to aid researchers and clinicians in developing new treatments and monitoring their effectiveness as well as reducing the time and cost of clinical trials(4, 6). MRI data were acquired according to the ADNI acquisition protocol. For each subject, we used the MRI scan from the baseline visit when available and from the screening visit otherwise.

Each scan was graded on several separate criteria: blurring/ghosting, flow artifact, intensity and homogeneity, signal-to-noise ratio (SNR), susceptibility artifacts, and gray-white/cerebrospinal fluid contrast. For each subject, we used the MRI scan considered to be the “best” quality scan by the ADNI investigators (12, 21, and 22).

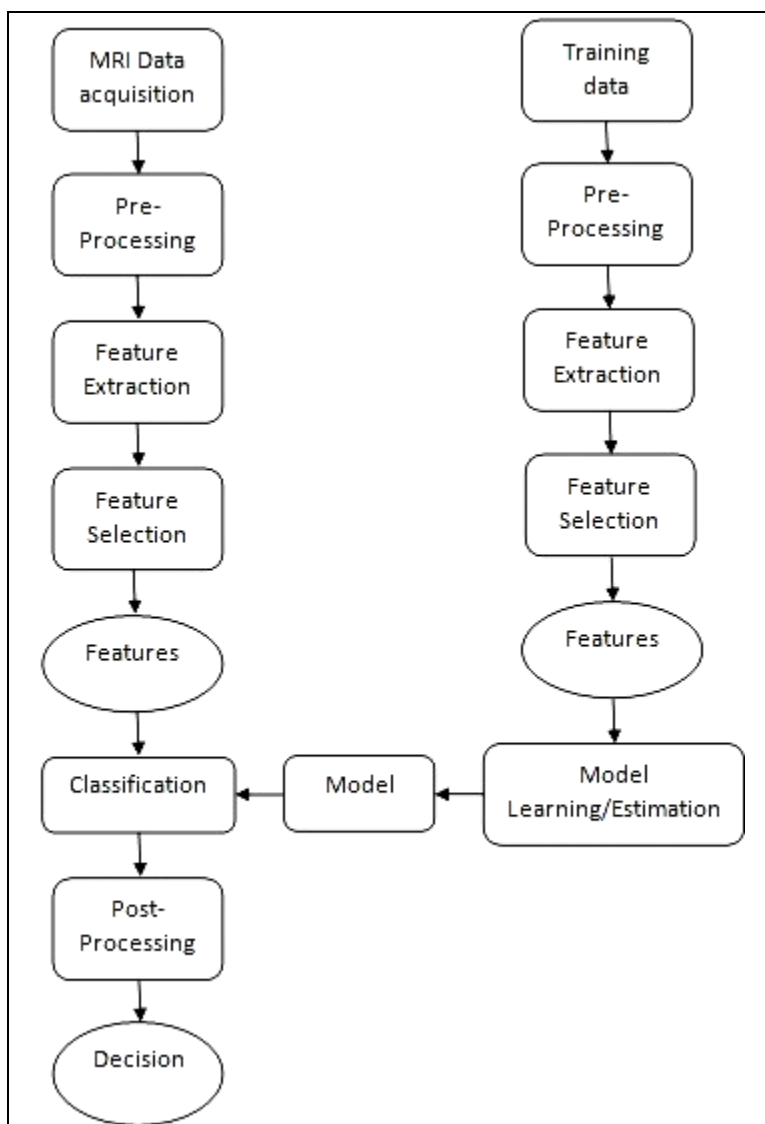
#### 2.2.2. Method

Pattern recognition consists of two major steps. First, features are extracted from the signals in such a way that they represent the signal very well. These features should contain all important information about the image. Then, according to a trade-off between required accuracy and computational cost, a smaller number of meaningful features are selected. The second step focuses on classification. A specific pattern is allocated to a class based on the characteristic features selected for it. In this proposal, the data are first pre-processed, which usually includes de-noising, image normalization,



and the isolation of patterns of interests from background. Then, during the feature extraction and feature selection phase, a group of appropriate

features are obtained. Finally using a good classifier, the data are classified.



**Figure 1.** Proposed research design.

In this proposal, preprocessing includes correction of inhomogeneities, registration to the stereotaxic space (e.g., using a linear transform), cross-normalization of the MRI intensity, de-noising, segmentation, and re-sizing of images using special software (e.g., SPM8 or MATLAB). Features (e.g., density) that can be extracted from the MRI include voxel-based, vertex-based, or ROI-based data. In the first category, the features are defined at the level of the MRI voxel. Specifically, the features are the probability of the different tissue classes (grey

matter, white matter, and cerebrospinal fluid) in a given voxel. In the second category, the features are defined at the vertex level on the cortical surface. Cortical thickness in the temporal, parietal, frontal, cingulate, precuneus, cuneus, and entorhinal cortices represents a direct index of atrophy and, thus, is a potentially powerful candidate for assisting in the diagnosis of AD.

Cortical thickness measures can be performed with the Free Surfer image analysis suite. The methods of the third category include only the

hippocampus. Their approach is based on the analysis of the volume and /or shape of the hippocampus. The hippocampus is affected at the earliest stages of the disease and, thus, can be used as a marker of early AD in a vast number of studies. Here, the segmentation of the hippocampus can be performed using SACHA.

Beginning with feature extraction steps, tissue segmentation (WM, GM, and CSF) can be performed by the SMP software. Image normalization is followed using a template. It may be followed by data modulation to maintain a constant total density. For bias correction purposes, customized tissue probability maps (TMPs) can be generated. Smoothing with a smoothing kernel/window is next. Then the image is re-sampled to an acceptable isotropic resolution. For feature reduction, a linear SVM can be used to reduce data to a subset of patterns. Down-sampling is achieved by simple averaging.

A threshold is set to remove CSF and voxels with less than certain intensity. A RIO template is created to remove discarded regions (e.g., cerebellum) from data sets. A linear SVM-based criterion might be used to carry out feature selection. By utilizing estimated weights, vector lengths are reduced. To overcome the disadvantage of the SVM-based approach, which ignores spatial information from adjacent voxels, a modified thresholding can be applied. Cross-validation can be used to achieve SVM parameter optimization. The algorithm is trained by some sub-groups and tested on others. Accumulating additional information and features does not necessarily lead to improved classification performance.

Augmenting the data size increases the dimensionality of the feature space, which can make the classifier unstable and lead to over fitting the data. This problem is well known in machine learning as the curse of dimensionality. However, advances in statistical learning with the development of new machine learning algorithms capable of dealing with high-dimensional data enable the development of new diagnostic tools.

In this study, down-sampling may be achieved via ROI-based measures instead of voxels as input features, principal component analysis, or partial least squares (PLS). In this proposal, we try to extract new features from structural MRI images and better classifiers (e.g., neural network) for automate classification between AD and other dementia. The newly proposed diagnostic criteria for AD must be validated in multiple large data sets, and we will evaluate our result by comparing them with reliable results from the ADNI's database

### 3. Benefits of the Study

#### 3.1. Benefits to science

Implementation of the proposed study will result in further progress in medical image classification as well as diagnostic imaging techniques used to early detect AD. The following scientific outcomes are expected:

- Implementation of new features from MRI imaging modality;
- Use of artificial intelligence and better classifier
- Improved accuracy of classification and early detection;
- Automated classification;
- In the near future, imaging and cerebrospinal fluid markers of amyloid deposition and glucose metabolism could be integrated with automated assessment of structural markers for optimal diagnosis and monitoring.

#### 3.2. Benefits to society

Improving techniques to detect and diagnose AD early will clearly have social and economic impacts on both healthcare professionals and those affected by AD and their family members. AD is currently diagnosed through longitudinal clinical evaluations, which are available only at specialized dementia clinics, making such evaluations; beyond the financial and geographic reach of most patients. Automated diagnosis tools that can be made available to community hospitals would therefore be very beneficial. Earlier identification of AD may enable earlier treatment and empower people to plan for their future sooner, including financial and legal matters such as:

- Reduction of patient trauma and improvement in quality of life;
- Reduction of the burden on caregivers;
- Access to training, education, and support services for caregivers and family members;
- Shortened terms of hospitalization and reduction of healthcare costs;
- Physicians' and caregivers' awareness of patients who may have difficulty managing their own healthcare, such as when and how to take other prescription medications;
- Reduced anxiety on the part of the affected person and his or her family about the cause of symptoms;
- Allows family members and caregivers to be alert to potential financial mismanagement and scams.

#### 4. Expected Results

It is expected that using structural MRI to predict AD during early stages will allow for diagnosis and treatment before irreversible neurodegeneration and functional impairment have occurred.

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#### References

- Saloni Tanna, Pharm.D. World Health Organization, Alzheimer's disease. Last updated October 7, 2004. accessed May 30, 2012 at: <http://archives.who.int/prioritymeds/report/background/alzheimer.doc>
- National Institute of ageing. Alzheimer's disease education and referral center. Available at: <http://www.alzheimers.org/generalinfo.htm#whatisad>
- Frisoni, G.B., et al., The clinical use of structural MRI in Alzheimer disease. *Nature Reviews Neurology*, 2010. 6(2): p. 67-77.
- Thies, W., Alzheimer's Association Report 2011 Alzheimer's disease facts and figures. *Alzheimer's & Dementia*, 2011. 7(2): p. 208-244.
- Weiner, M.W., et al., The Alzheimer's Disease Neuroimaging Initiative: A review of papers published since its inception. *Alzheimer's and Dementia*, 2011.
- Petersen, R., et al., Alzheimer's Disease Neuroimaging Initiative (ADNI). *Neurology*, 2010. 74(3): p. 201-209.
- Small, G.W., et al., Current and future uses of neuroimaging for cognitively impaired patients. *The Lancet Neurology*, 2008. 7(2): p. 161-172.
- Zhang, D., et al., Multimodal classification of Alzheimer's disease and mild cognitive impairment. *Neuroimage*, 2011.
- Teipel, S.J., et al., Multivariate network analysis of fiber tract integrity in Alzheimer's disease. *Neuroimage*, 2007. 34(3): p. 985-995.
- Tondelli, M., et al., Structural MRI changes detectable up to ten years before clinical Alzheimer's disease. *Neurobiology of Aging*, 2011.
- Liu, Y., et al., Analysis of regional MRI volumes and thicknesses as predictors of conversion from mild cognitive impairment to Alzheimer's disease. *Neurobiology of Aging*, 2010. 31(8): p. 1375-1385.
- Wolz, R., et al., Multi-Method Analysis of MRI Images in Early Diagnostics of Alzheimer's Disease. *PloS one*, 2011. 6(10): p. e25446.
- Jeffrey R. Petrella, et al, Neuroimaging and early diagnosis of Alzheimer disease: A Look to the future. 10.1148/radiol.2262011600 February 2003 *Radiology*, 226, 315-336.
- Bozzao A, et al. Diffusion and perfusion MR imaging in cases of Alzheimer's disease: correlations with cortical atrophy and lesion load. *AJNR* 2001 ;22:1030 –1036
- Kantarci K, et al. Mild cognitive impairment and Alzheimer disease: regional diffusivity of water. *Radiology* 2001 ;219:101 –107
- Joseph F. Norfray, et al. Alzheimer's Disease: Neuropathologic Findings and Recent Advances in Imaging. *AJR* January 2004 vol. 182 no. 1 3-13
- Westman, E., et al., AddNeuroMed and ADNI: Similar patterns of Alzheimer's atrophy and automated MRI classification accuracy in Europe and North America. *Neuroimage*, 2011.
- Davatzikos, C., et al., Prediction of MCI to AD conversion, via MRI, CSF biomarkers, and pattern classification. *Neurobiology of Aging*, 2010.
- Ritchie, K. & Lovestone, S. The dementias. *Lancet* 360, 1759–1766 (2002)
- Hampel, H., et al., Biomarkers for Alzheimer's disease therapeutic trials. *Progress in Neurobiology*, 2011. 95(4): p. 579-593.
- Cuingnet, R., et al., Automatic classification of patients with Alzheimer's disease from structural MRI: A comparison of ten methods using the ADNI database. *Neuroimage*, 2011. 56(2): p. 766-781.
- Dubois, B., et al., DTI and Structural MRI Classification in Alzheimer's Disease. *Advances in Molecular Imaging*, 2012. 2.
- Jalalian, Mehrdad. Writing for academic journals: A general approach. *Electronic Physician Journal*. 2012; 4(2): 476-477. Available online at: <http://www.ephysician.ir/2012/476-477.pdf>

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