CONTENTS

In vivo Efficacy of Lactic Acid Bacteria in Biological Control against *Fusarium oxysporum* for Protection of Tomato Plant

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Abstract: Lactic acid bacteria (LAB), isolated from milk and yoghurt, were tested for their efficacy against some phytopathogenic fungi under *in vitro* and *in vivo* tests. *Fusarium oxysporum*, one of most important pathogenic fungi invade tomato plants, was chosen to evaluate the effectiveness of LAB as a biocontrol agent under *in vivo* tests. Culture broth of LAB was applied as seed treatment or soil drench. The protective effect of LAB significantly increased after challenging inoculation by *F. oxysporem*, especially when LAB were applied as seed treatment; the number of roots increased by 216, 311, and 358% over control with LB-1, LB-4, and LB-5, respectively, whereas the increment was 169, 163, and 181% for soil drench. Interestingly, when LAB were applied as seed treatment, in soil infested with *F.oxysporum*, the total fresh weight of tomato plants increased by 348, 260, and 390% with LB-1, LB-3, LB-5, respectively, whereas the increment was 268, 427, and 393% with LB-1, LB-4, and LB-5, respectively, for soil drench. Overall, while previous reports of antifungal activity by LAB under *in vitro* tests are scarce, we have demonstrated for the first time the capability of LAB to act as plant growth promoting bacteria and biocontrol agent against some phytopathogenic fungi under *in vivo* tests.

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Keywords: Lactic acid bacteria, biocontrol, plant growth promoting, plant pathogenic fungi, *Fusarium oxysporum*.

1. Introduction

Soil-borne fungal diseases are among the most important factors limiting the yield of many economically important plants, resulting in serious economic losses. Several soil-pathogens attack roots and shoots of plants, causing damping-off or root-rot (**Elad *et al.*, 1982; Thomasho, 1996**). Chemical fertilizers and pesticides are extensively used to prevent or control plant diseases. However, the environmental pollution caused by excessive use of such agrochemicals as well as the development of resistant pathogens have promoted the search for alternative approaches, i.e. the use of microorganisms or their metabolites (**Ronel *et al.*, 1986; Montesinos, 2003**). It has been established that different species of bacteria and fungi such as *Trichoderma*, *Pseudomonas*, *Stryptomyces*, and *Bacillus* are used as antimicrobial agents against phytopathogenic fungi. Recently, LAB have received much attention. Application of LAB in traditional food and feed fermentation and preservation is well documented (**Kim, 1993; Johan and Jesper, 2005**). LAB are harmless and used to improve human and animal health (probiotics). LAB have a GRAS state (generally recognized as safe) and it has been estimated that 25% of the European diet and 60% of the diet in many developing countries consist of fermented foods (**Stiles, 1996**). LAB produce a variety of antimicrobial compounds and effective substances such as lactic, acetic, probionic acids, antibiotics, bacteriocins as well as hydrogen peroxide and carbon dioxide (**Ouwehand, 1998**). The precise mechanism of antimicrobial action is difficult to elucidate due to the complex and commonly synergistic interactions between different compounds (**Corsetti *et al.*, 1998**). However, the mechanism can be attributed to both competition for nutrient and production of antibiotics and various inhibitory substances (**Johan and Jesper, 2005**). Many of the antimicrobial compounds affect the physiological activities of a pathogen such as cell division, biosynthesis of DNA, RNA, protein, lipid metabolism and cell synthesis (**Chaurasia *et al.*, 2005**). The effect of antibiotics and other compounds produced by LAB has widely been researched, especially in fermented foods (**Johen and Jesper, 2005**; **Lindgren and Dobrogosz, 1990),** silage (**Stirling and Whittenbury, 1963; Woolford, 1994**), and as biopreservatives (**Johan and Jesper, 2005**).

During the past 50 years, many studies were reported about bacterial and fungal plant diseases as well as the application of different microorganisms as biocontrol agents. However, no information is available on the interactions of LAB with phytopathogenic fungi (**Stephane *et al.*, 2005; Ashgar and Mohammad, 2010**). Very few *in vitro* studies have been reported about the efficacy of LAB

against phytopathogenic fungi (**Zulpa *et al.*, 2003; Rosalia *et al.*, 2008; Wang *et al.*, 2011**).

The aim of our contribution was to: 1) evaluate the influence of some strains of lactobacilli against some phytopathogenic fungi under *in vitro* tests , 2) Assess the efficacy of LAB to act as plant growth-promoting bacteria (PGPB) for tomato plants under *in vivo* tests, and 3) evaluate the potential of LAB against *F. oxysporum* in pot trials, using culture broth of LAB applied as seed treatment or soil drench. To the best of our knowledge, this is the first study dealt with the potential of LAB against the fungus *F. oxysporum* under *in vivo* tests.

2. Material and Methods

Lactic acid bacteria for antifungal activity

Lactic acid bacteria (LAB), included *Lactobacillus* sp. 1 (LB-1), *Lactobacillus acidophilus* sp. 2 (LB-2), *Lactobacillus* sp.4 (LB-4), *Lactobacillus* sp.5 (LB-5), were previously isolated from yoghurt and milk. In addition, a lyophilized strain of *Lactobacillus plantarum* NRRL B-4524 (LB-3) was from the National Center for Agricultural Utilization Research (USA). All strains were kept on MRS agar (Van den Berg *et al.*, 1993). Fresh cultures are grown in MRS broth at 30^{o}C for 24hrs before use in experiments.

Phytopathogenic fungi

High virulent strains of pathogenic fungi, previously isolated from diseased plants, were used in this study. *Fusarium oxysporum*-1 (isolated from tomato), *Rhizoctonia solani*-1 (isolated from lupine), *F.oxysporum*-2 (isolated from cotton), *Rhizoctonia solani*-2 (isolated from tomato), and *Sclorotium rolfsii* (isolated from onion). Fungal strains were maintained on potato dextrose agar (PDA) at 4^{o}C.

Preparation of spore suspension

F. oxysporum-1 was grown in PDB at 28^{o}C. After incubation for 7 days, fungal biomass was homogenized in a blender for one minute. Spore suspensions were prepared using sterile distilled water to a concentration of 5x10^{5} spores. The spore suspension was prepared just before each experiment.

In vitro assays

In vitro assays for antifungal activity by LAB were determined using conical flasks (250-ml) containing PDB as the growth medium for all test fungi. Flasks, supplemented with LAB, were inoculated with the test fungi and incubated at 28^{o}C for each of all fungal strains. After incubation for 7days, fungal growth were filtered, washed several times with distilled water, and dried at 55^{o}C to a constant weight. Percentage of growth inhibition (GI) was calculated using the formula: GI (%) = $C_0 - C_F / C_o$ x 100%, where C_0 is the dry weight of fungal mycelium (control), C_F is the dry weight of fungal mycelium after inhibition by LAB.

In vivo experimental design

Preparation of Tomato seedlings

Peat moss soil was dispensed into plastic trays (160 eyes) for growth of seedlings. Moisture content of peat moss was sustained at a proper level throughout seedlings growth. Tomato seeds (UC 97) were divided into two groups: in one group, seeds were soaked in culture broth of LAB strains for 1 hr; 1ml for one seed was applied. The second group included non-soaked seeds. After 45 days, healthy seedlings were transplanted for pot trials.

In pot trials

All experiments were performed in pots, 30 cm diameter, filled with unsterilized natural soil. Uninoculated or inoculated soil with *F.oxysporum*-1, at a rate of 10-ml homogenized culture per pot, were prepared one day before planting. Two treatments were performed: firstly, in which tomato seeds were pre-soaked in culture broth of LAB, resultant seedlings were placed in pots uninoculated or inoculated with *Fusarium*, without supplementation of soil with LAB (seed treatment). Secondly, where non-soaked seeds were used, seedlings were also placed in pots in absence and presence of *Fusarium* with supplementation of soil with LAB, at a rate of 10-ml culture broth per pot (soil drench). All experiments and controls were replicated three times. Plants were drenching at intervals and grown for another 45 days. *In vivo* tests were performed during 3-months, from April up to June, where the atmospheric temperature ranged from 25 - 38^{o}C.

Sampling and analysis

After three months growth, tomato plants were harvested. To reveal the effect of LAB on the growth characteristics, each plant was measured for shoot- and root-length, number of secondary roots, and total fresh weight of plants.

Statistical analysis

Data were analyzed using SPSS for windows (SPSS Inc.) by means of a one-way ANOVA and subsequently differences between treatments were determined using least significant differences (LSD at α 0.05).

3. Results and Discussion

In vitro efficacy of LAB against phytopathogenic fungi

Results of *in vitro* inhibition by LAB revealed that fungal strains behaved differently. Low**,** moderate, to high inhibition effects were observed by LAB against the pathogenic fungi. Low inhibition effect was observed by all strains of LAB against *F.oxysporum*-1, whereas both of *R.solani*-1 and *F.oxysporum*-2 were moderately inhibited by LB-2, LB-3, and LB-4 (Fig. 1). High inhibition effect was observed against *S. rolfsii*; it was highly inhibited by 86, 84, and 75% with LB-2, LB-3, LB-4, respectively. The only exception as negative impact by LAB was observed with LB-1 which showed growth promotion for the fungus *R.solani-2;* the dry weight of the fungal mycelium reached 114*%* over control (inhibition 0%, Fig.1). Thus, *in vitro* studies revealed that *S. rolfsii* was the most inhibited pathogen by LB-2, LB-3 and LB-4, whereas LB-1 and LB-5 showed the lowest inhibition effect against most of the tested pathogens. In addition, *F.oxysporum*-1 was the most resistant fungus to all the strains of LAB under *in vitro* tests. We have found very few references about *in vitro* effect of LAB as biocontrol agents against phytopathogenic fungi. **Zulpa *et al.* (2003)** reported that the inhibitory effect of extracellular products of *Streptococcus thermophilus* against "wood blue stain fungi" could be attributed to antimicrobial substances such as lactic acid ,organic acid ,and hydrogen peroxide. **Rosalia *et al.* (2008)** have reported that, LAB isolated from fresh fruits and vegetables, were found to produce organic acid substances that affected some phytopathogenes, causal of postharvest. **Wang *et al.* (2011)** found that, metabolites of *Lactobacillus plantarum* IMAU10014 possess high activity against some plant pathogenic fungi and that the antifungal effect is attributed to a proteinaceous substance. In our study, growth inhibition of phytopathogenic fungi by LAB using PDB medium indicated that, even in competition with the pathogen under conditions more favorable to the pathogen, reduction in fungal growth was observed.

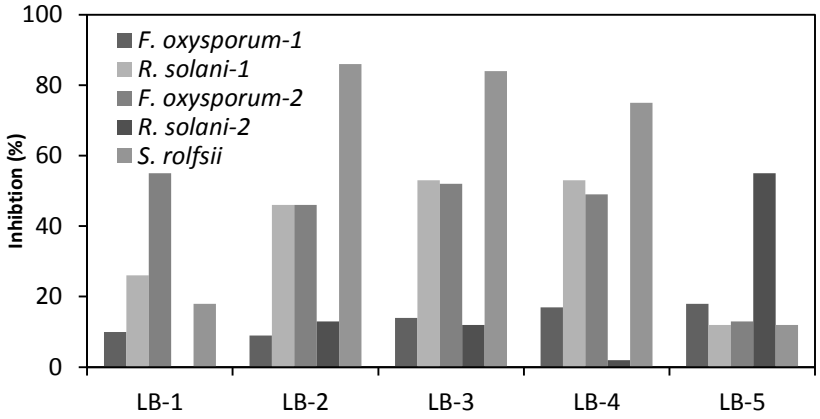

Fig.1. The percentage of growth inhibition of phytopathogenic fungi by lactic acid bacterial strains; LB-1, LB-2, LB-3, LB-4, and LB-5.

The inhibitory effect of LAB may also be attributed to the production of antibiotics, especially nisin (Yomna, 2000) as well as competition for nutrients as indicated above. On other hand, the low inhibition effect by LAB against *F.oxysporum*-1 may be elucidate by an assumption that the concentration of the produced inhibitors may not be high enough to inhibit the fungal growth. The opposite effect (114% growth promotion) occurred by LB-1 with *R. solani*-2 (Fig.1) may be attributed to the growth-promoting effect for a pathogen , i.e. some bacterial strains produce opposite effects (growth promotion or inhibition) depending on the sensitivity threshold of the fungus to the extracellular metabolites produced by bacteria (**Don *et al.*, 1993; Zulpa *et al.*, 2003**).

***In vivo* efficacy by lactic acid bacteria**

Being the most resistant fungus to all strains of LAB under *in vitro* tests, *F.oxysporum*-1 was chosen for *in vivo* tests using tomato seeds.

Plant growth-promoting effect by lactic acid bacteria

In absence of *Fusarium*, results of pot trials with LAB applied as seed treatment or soil drench revealed their ability to enhance plant growth compare with control (Fig. 2).

However, the plant growth characteristics significantly differed in response to LAB strains. When LAB was applied as seed treatment, the shoot length was 28.00 and 37.67cm (112 and 151% over control) with LB-1 and LB-5, respectively, with no obvious increase in root length (Table 1). However,

the number of secondary roots increased by 48, 41, and 62 (171, 146, and 221% over control) with LB-1, LB-3, and LB-5, respectively. On other hand, when LAB were applied as soil drench, the shoot length was 32.50, 34.33, and 35.00 cm (135, 143, and 146% over control) with LB-1, LB-2, and LB-5, respectively (Table 1); whereas, number of secondary roots increased by 36, 35, and 42 (164, 159, and 191% over control) with LB-1, LB-2, and LB-5, respectively (Table 1). These results reveal the capability of LAB to be considered as PGPB. Application of PGPB has been hampered by inconsistent performance in field tests; this is usually attributed to their poor rhizosphere competence (**Thomashow, 1996**). Rhizosphere competence of biological control agents comprises effective root colonization combined with the ability to survive and proliferate along growing plant roots over a considerable time period in presence of the indigenous microflora (**Stephane *et al.*, 2005**).

Fig. 2. Plant growth promoting effect of lactic acid bacteria for tomato plant compare with control, 0 (left image; plant without bacteria or fungi). Culture broth of lactic acid bacteria was applied as seed treatment (middle image) or soil drench (right image). Plants were harvested after 3-months growth.

Table 1. Mean shoot- and root-length (cm), and number of secondary roots per root system of tomato plants, treated with lactic acid bacteria, in soil non-infested with *Fusarium*.

LAB	Seed treatment			Soil drench		
	Shoot length	Root length	S-roots*	Shoot Length	Root length	S-roots*
Control	25.00^{ab}	14.50^{a}	28.00	24.00^{ab}	15.50^{b}	22.00
LB-1	28.00^{f}	14.33^{fg}	48.00	32.50^{de}	19.00^{b}	36.00
LB-2	20.17^{j}	12.67^{hg}	39.00	34.33^{cd}	18.67^{b}	35.00
LB-3	25.33^{g}	15.33^{ef}	41.00	12.17^{ij}	12.67^{gh}	22.00
LB-4	16.83^{k}	11.50^{h}	25.00	27.67^{f}	14.00^{fg}	29.00
LB-5	37.67^{b}	14.33^{fg}	62.00	35.00^{c}	31.00^{a}	42.00

*S-roots = number of secondary roots, one value was chosen from three replicates.

Our results could confirm efficacy of LAB as PGPB; bioprotection of tomato seeds and the soil drench with LAB support plant growth, especially that a single application by LAB was used. It seems that nutrient availability for tomato plants is not limited despite of long duration (3-months of seed treatment or 45 days of soil drench). **Lewis and Papaviza (1984)** reprted that, *Trichoderma harzianum* required a constant nutrient supplementation for optimum biocontrol efficacy against phytopathogenic fungi. **Rose *et al.* (2003)** reported that, two applications of the biocontrol agents *Trichoderma harzianum*, *Pseudomonas chlororaphis* and *Streptomyces griseoviridis* were needed every ten days for effective controlling against *F. oxysporum*.

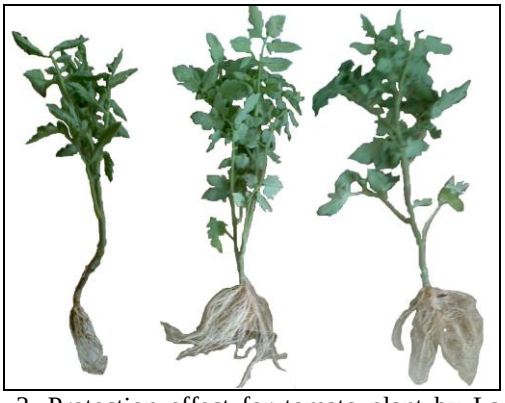

Fig. 3. Protection effect for tomato plant by Lactic acid bacteria as biocontrol agent against *F. oxysporum*-1 (control plant+fungi, left image). Culture broth of lactic acid bacteria was applied as seed treatment (middle image) or soil drench (right image). Plants were harvested after 3-months growth.

Antifungal activity by lactic acid bacteria

LAB could be considered as PGPB as indicated above. The protective effect of LAB significantly increased after challenging inoculation of soil with *F. oxyspoium*-1 (Fig. 3). Moreover, plant measurements and the number of secondary roots significantly increased with all strains of LAB (Table 2). When LAB were applied as seed treatment, the number of secondary roots increased by 41, 59, and 68 (216, 311, and 358% over control) with LB-1, LB-4, and LB-5, respectively (Table 2). When soil drench was applied by LAB, the number of secondary roots was 27, 26, and 29 (169, 163, and 181% over control) with LB-1, LB-4, and LB-5, respectively.

Moreover, the total fresh weight (TFW) of tomato plants increased about 2-4 fold with LAB in soil infested with *Fusarium* (Table, 3). The TFW of tomato plants reached 27.37, 20.50, and 30.67g (348, 260, and 390% over control) with LB-1, LB-3, and LB-5, respectively, for seed treatment, whereas it reached 21.13, 33.60, and 30.90g (268, 427, and 393%) with LB-1, LB-4, and LB-5, respectively, for soil drench. A possible mechanism for increasing plant growth and TFW by LAB may be due to the efficiency in nutrient transfer from soil to the roots and plants as a result of increasing the number of roots and bioprotection of rhizosphere area by LAB.

Another mechanism for increasing plant growth and TFW are caused by the antifungal metabolites (antibiotic) produced by LAB against *Fusarium*, especially when synergistic effects of lytic enzymes of fungal cell wall, produced by other potential antagonists are thought, leading to the enrichment of soil with nutrients.

Table 2. Mean shoot- and root-length (cm), and number of secondary roots per root system of tomato plants, treated with lactic acid bacteria, in soil infested with *Fusarium*.

LAB	Seed treatment			Soil drench		
	Shoot length	Root length	S-roots*	Shoot Length	Root length	S-roots*
Control	20.60^{ab}	11.70^{b}	19.00	21.00^{b}	14.50^{b}	16.00
LB-1	24.00^{gh}	18.67^{b}	41.00	31.33^{e}	18.67^{b}	27.00
LB-2	23.67^{gh}	13.00^{gh}	35.00	27.50^{f}	15.67^{ef}	21.00
LB-3	28.10^{f}	16.50^{de}	38.00	22.67^{hi}	16.67^{cde}	21.00
LB-4	31.00^{e}	15.00^{ef}	59.00	31.67^{e}	18.17^{bcd}	26.00
LB-5	38.50^{b}	15.33^{ef}	68.00	44.50^{a}	18.33^{bc}	29.00

*S-roots = number of secondary roots, one value was chosen from three replicates.

In addition, treatment of soil with LAB may trigger systematic acquired resistance (SAR) which develops when plants successfully activate their defense mechanism, in presence of a pathogen infection, resulting in an enhanced synthesis of plant defense chemicals which support plant growth and fortify plant cell wall strength (**Stephane *et al.*, 2005**). In the present study, the obvious elongation in both shoot- and root-length and the increasing number of secondary roots (Fig. 3) as well as the increment in TFW of tomato plants (Table 3) may confirm the capability of LAB to trigger the SAR of tomato plants and induce the production of growth regulators, stimulants, or hormones by which elongation of plant and increment of TFW are occurred (Figs. 2 and 3). Worthy mention is that, LB-1 and LB-5 showed higher antifungal activity under *in vivo* tests, which is contrary to the results of *in vitro* tests. Actually, *in vivo* tests ensure the efficacy of LAB as biocontrol agent against phytopathogenic fungi, indicating that *in vitro* assays are not fully predictive for the inhibitory action confirmed under *in vivo* tests against a pathogen (**Faina *et al.*, 2007**).

Table 3. Mean total fresh weight (g) of tomato plants as affected by treatment with LAB and infection with *F.oxysporum*-1.

LAB	Seed Treatment		Soil Drench	
	Soi l w/o *Fusarium*	Soil with *Fusarium*	Soil w/o *Fusarium*	Soil with *Fusarium*
LB-1	21.00^{e}	27.37^{c}	20.03^{e}	21.13^{e}
LB-2	8.77^{j}	12.83^{gh}	17.77^{f}	12.27^{hi}
LB-3	21.87^{de}	20.50^{e}	13.67^{gh}	12.37^{ghi}
LB-4	10.67^{ij}	9.53^{j}	14.50^{g}	33.60^{a}
LB-5	34.73^{a}	30.67^{b}	23.60^{d}	30.90^{b}

Total fresh weight of control (plant+fungi) = 7.87^{b}. The control value was set as 100%.

The antifungal effect of LAB under *in vivo* conditions may lead us for thinking about the efficacy of LAB as root colonizer. We can't exclude the possibility that on roots, LAB could attack, colonize, and reduce fungal growth much faster than under *in vitro* tests.

LAB are used for preservation of food and milk products from centuries and acquired the GRAS status. Thus, metabolic products of LAB can be safely used in biocontrol of plant pathogenic fungi.

The use of LAB as biocontrol agent against phytopathogenic fungi presents both challenges and opportunities for management of plant diseases. We demonstrated for the first time the efficacy of LAB as biocontrol agent under *in vivo* conditions. The success of biological control in our tests is surprising compare with the control. LAB may produce a variety of antifungal substances under *in vitro* tests, but under *in vivo* tests the mechanism of antifungal action is difficult to elucidate due to the complex and commonly synergistic interactions between different compounds and different soil microbiota (**Naseby *et al.*, 2000; Johan and Jesper, 2005**). It could also be suggested that, a synergistic effect of LAB with other beneficial microorganisms in soil may provide an almost constant nutrient source for the plants. Other potential antagonists in soil may secrete hydrolytic enzymes that degrade *Fusarium* cell wall and produce exopolysaccharides which may contribute for enrichment of soil with nutrients, leading to the plant healthy. *In vivo* tests, however, showed LAB to be considered as biocontrol agent against *F.oxysporum* despite of stress conditions such as fluctuation in temperature, relative humidity, and a greater variety of competitive microorganisms.

The more practical approach has been used in our study is to elucidate the efficacy of LAB, isolated from milk and yoghurt, for biocontrol against phytopathogenic fungi. LAB with antifungal and antibacterial activity are well documented in food, meat, and milk products as biopreservatives (**Johen and Josper, 2005**), while less attention has been paid to exploit the antifungal activity of LAB for biocontrol of phytopathogenic fungi. When this effect was reported, under *in vitro* assays, it was attributed to the production of indol acetic acids and phenolic substances (**Zulpa *et al.*, 2003**), organic acids (Rosalia et al. 2008), or proteinaceous compounds (**Wang *et al.*, 2011**). The antifungal substances produced by LAB in this study are antibiotics (**Yomna, 2000**). However, competition for nutrient and space for preventing the pathogen to colonize the rhizosphere may also be another mechanism.

Conclusion

Bioprotection of tomato plants by LAB isolated from yoghurt and milk is a finding reinforced not only by their inhibition capacity but also by their persistance in soil under hard conditions. LAB seem to be more resistant to stress conditions such as fluctuation in temperature and relative humidity. Furthermore, each strain of LAB showed antifungal activity towards more than one pathogen, under *in vitro* tests. There was no distinct correlation between the *in vitro* positive antagonism and the *in vivo* positive antagonism. The use of chemicals and fungicides in agriculture as well as the environmental pollution would be avoided by LAB as a promising PGPB and biocontrol agent. Future research should confirm the mechanism of inhibition, assay for lytic enzymes, and determination of inhibitor substances other than antibiotics for application of LAB in biocontrol as a viable alternative method to manage plant diseases.

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References

Ashgar, H Mohammad, P. (2010). A review on biological control of fungal plant pathogens using microbial antagonists. J Biolog Sci., 10:273-290.

Chaurasia, B Pandey, M Palni, M *et al.* (2005) Diddusible and volatile compounds produced by an antagonistic Bacillus subtilis strain couse structural deformations in pathogenic fungi *in vitro*. Microbios Res., 160:75-81.

Corsetti, A Gobbetti, M Rossi, J Damiani, P. (1998). Antimould activity of sourdough lactic acid bacteria: identification of a mixture of organic acids produced by Lactobacillus sanfrancisco CBI. Appl Microbiol Biotechnol., 50:253-256.

Don, L James, M John, M Margaret, A (1993). Isolation and characterization of actinomycete antagonists of a fungal root pathogen. Appl Env Microbiol., 59:3899-3905.

Elad,Y Kalfan, A Chet, I (1982) Control of Rhizoctonia solani in cotton by seed-coating with Trichoderma spp. spores. Plant soil, 66:279-281.

Faina,K Johan, H Ben, L (2007). Collimonas fungivorans, an unpredicted in vitro but efficient *in vivo* biocontrol agent for the suppression of tomato foot and root rot. Env Microbiol., 9:1597-1603.

Johan, S Jesper, M (2005) Antifungal lactic acid bacteria as biopreservatives.Trends Food Sci Tech., 1:70-78.

Kim, WJ (1993). Bacteriocins of lactic acid bacteria: their potentials as food preservatives. Food Rev Int., 9:299-313.

Lewis, J. Papaviza,G. (1984). A new approach to stimulate population proliferation of Trichoderma species and other potential biocontrol fungi introduced into natural soils. Phytopathology, 74, 1240-1244.

Lindgren, S Dobrogosz,W (1990). Antagonistic activities of lactic acid bacteria in food and feed fermentations. FEMS Microbiol Rev., 87:149-164.

Montesinos, E (2003) Development, registration and commercialization of microbial pesticides for plant protection. Int. Microbiol., 6: 245-252.

Naseby, D Pascual, J Lynch, J (2000). Effect of biocontrol strains of Trichoderma on plant growth, Pythium ultimum populations, soil microbial communities and soil enzyme activities. J Appl Microbiol., 88:161-169.

Ouwehand, A (1998). Antimicrobial components from lactic acid bacteria. In Lactic acid bacteria Microbiology and Functional Aspects ed Salminen, S Von Wright A., pp.139-159.New York:Marcel Dekker Inc.

Ronel,V Wilhelm, H Johannes, J Kotze, J (1986). Antagonism of lactic acid bacteria against phytopathogenic bacteria.Appl Envir Microbiol., 52: 552-555.

Rosalia,T Lluis, B Emilio,M Esther, B (2008) . Lactic acid bacteria from fresh fruit and vegetables as bioontrol agents of phytopathogenic bacteria and fungi. Int Microbiol., 11:231-236.

Rose, S., Parker, M. (2003). Efficacy of biological and chemical treatments for control of Fusarium root and steam rot on greenhouse Cucumber. Plant Disease, 87, 1462-1470.

Stephane, C Brion, D Jerzy, N Christophe,C Essaid, A (2005). Use of plant growth-promoting bacteria for biocontrol of plant diseases:principles,mechanism of action, and future prospects.Appl Envir Microbiol., 71: 4951-4959.

Stiles, ME (1996). Biopreservation by lactic acid bacteria. Antonie van Leeuwenhoek, 70: 333-345.

Stirling, A Whittenbury, R (1963). Source of the lactic acid bacteria occurring in silage. J. Appl. Bacteriol., 26: 86-90.

Thomashow, LS (1996). Biological control of plant root pathogens. Curr Opin Biotechnol., 7:343-347.

Van den Berg, E Annelies, S Bruno, P *et al.* (1993) Isolation, screening and identification of lactic acid bacteria from traditional food fermentation processes and culture collections. J Food Biotechnol., 7: 189-205.

Wang, H Yan, H Shin, J *et al.* (2011). Activity against plant pathogenic fungi of *Lactobacillus plantarum* IMAU10014 isolated from Xinjiang Koumiss. Ann. Microbiol., 61, 879-885. Doi: 10.1007/ s 13213-011-0209-6.

Woolford, MK (1994). The antimicrobial spectra of some salts of organic acids and glutaraldehyde in respect to their potential as silage additives. Grass and Forage Science, 39:53-57.

Yomna, A Mostafa (2000). Microbiological and biochemical studies on the production of antibiotic (nisin) from some strains of lactic acid bacteria. MS Thesis Ain Shams Univ ., pp.1-121.

Zulpa, G Zaccaro, M Boccazzi, F Parada, J Storni,M (2003). Bioactivity of intra and extracellular substances from cyanobacteria and lactic acid bacteria on"wood blue stain" fungi. Biological control, 27:345-348.

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Detection of Brucella spp. and vaccine strains in bovine aborted fetuses by a multiplex PCR

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Abstract: The purpose of this study was to determine the seroprevalence of brucellosis among aborted cows and simultaneously to detect bacterial DNA in their aborted fetal tissues by means of PCR. In this descriptive study, peripheral blood samples were drawn from 76 cows aborted at the dairy farms of Tabriz (North-West of Iran) and their sera separated by centrifugation. The serum samples were analyzed by ELISA (Pourquire-ELISA Kit manufactured by France). Consequently, tissue samples were taken from the stomach (fluid), liver, kidney, spleen, lungs, heart and placenta of aborted fetuses and tested by PCR. Six out of 76 dams (7.8 percent) were seropositive to the Brucella spp., and six out of 76 aborted fetuses (7.8 percent) showed a positive reaction to the PCR test. Four out of six aborted fetuses (66 percent) showed a positive reaction against the Brucella abortus and the two remaining (34 percent) had a positive reaction to the vaccine strain, RB51. Statistical analysis did not show any significant difference between the two diagnostic methods (PCR and serological tests). However, PCR protocol is preferred to the serological tests due to its ability to differentiate among the Brucella strains. In conclusion, both serological and particularly PCR tests are recommended for diagnosis of Brucella strains in cows subjected to abortion and according to our PCR test results, vaccination with RB51 strain could be abortive in some pregnant cows.
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Keywords: Abortion; Brucellosis; Cow; PCR; ELISA

1. Introduction

Brucellosis of cattle, also known as contagious abortion and Bang's disease, is caused by infection from the bacterium Brucella abortus, which can also cause a disease of humans known as undulant fever (Radostitis et al., 2007). This disease causes abortion or premature calving of recently infected animals, most often between the fifth and eighth month of pregnancy. Infected cows frequently suffer from retained afterbirth, are difficult to get rebred, and are sometimes left sterile. The organism has an affinity for the reproductive tract and is spread from the vaginal discharge of an infected cow or from an aborted fetus. Infected breeding bulls can transmit the disease to cows at the time of service by infected semen (Noakes et al., 2009).

Because of the enormous losses the disease causes to the Iranian dairy and beef cattle industries (primarily due to abortion in the second half of gestation) correct and prompt diagnosis is important in controlling and eradicating the disease in this region. Efficient diagnosis requires a complete diagnostic protocol associated with submission of appropriate specimens and clinical history. Traditional diagnostic tools include serology, histopathology, bacterial isolation, and for certain agents direct examination or immunohistochemistry (Anderson, 2007). Direct methods based on bacteriological isolation are usually employed, but they are difficult, time consuming and dangerous (Richtzenhain, et al., 2002).

Since the early 1990s, PCR has been increasingly used as a diagnostic tool for etiologic diagnosis of abortion in cattle either as a complement or replacement of time consuming traditional diagnostic methods (Anderson, 2007). Several PCR and Rt-PCR (reverse transcription-polymerase chain reaction) protocols have been recently developed for identification of infectious agents in aborted bovine fetuses, including Brucella abortus (Leal-Klevezas et al., 2000; Cortez et al., 2001; Richtzenhain et al., 2002; Bricker et al., 2003). Considering the potential of PCR for etiological diagnosis of infectious bovine abortion, the objective of this study was to use PCR protocol as a tool for identification of Brucella spp. in tissues from aborted bovine fetuses and simultaneously ELISA as a complementary test for detection of Brucella antibodies in sera of dams subjected to abortion. This study included frozen tissues from aborted fetuses and sera from aborted dams.

2. Material and Methods

2.1 Samples

From May 2008 through August 2010, 76 blood and tissue samples were collected from aborted cows and their fetuses (n=76) at the dairy farms located in the Tabriz vicinity. Blood samples were centrifuged and sera harvested and kept at -20°C. Tissue samples were collected from several fetal organs including liver, kidney, lung, spleen, heart, stomach fluid and placenta, then separately

pulverized under liquid nitrogen and finally stored at -20°C until DNA extraction.

2.2 Enzyme- Linked Immunosorbent Assay (ELISA)

Using a commercial ELISA kit (Pourquier, France), sera were tested for the presence of antibodies to Brucella abortus according to manufacturer's instruction.

2.3 DNA extraction

DNA extraction from frozen tissues samples was performed using a commercial kit (Accuprep Genomic DNA Extraction Kit, Bioneer, S. Korea) following the manufacturer's instructions. Briefly, 100µL of thawed homogenates of fetal tissues were mixed with 600µL of Nuclei Lysis Solution and homogenized for 10 seconds. Samples were incubated at 65°C for 30min, followed by the addition of 17.5µL proteinase K (20mg mL-1) and incubation at 60°C for three hours, vortexing every 30 min. Three microliters of RNase A (4mg mL-1) were added, the samples were mixed and incubated at 37°C for 30minutes. After cooling, 200µL of Protein Precipitation Solution were added, followed by vortexing and centrifugation at 13,000 g for 4minutes. The supernatant was transferred to a new microtube with 600µL of isopropanol, mixed, and centrifuged at 13,000 g for 3minutes. The supernatant was discarded and the pellet was washed with 600µL of 70% ethanol, followed by a final centrifugation at 13,000 g for 3min. Each pellet was dissolved in 100µL of DNA Rehydration Solution by incubating at 65°C for one hour.

DNA quality was assessed by spectrophotometry and PCR amplification of an internal control (prolactin gene). Samples that did not yield a prolactin amplicon nor had DNA concentration lower than 100ng µL-1 as assessed by spectrophotometry were excluded from further analysis.

Table 1. Primer sequences for different Brucella species and strains.

No	Bacterial name	Primer sequence	PCR product molecular weight (bp)
1	*Brucella abortus*,	5-AGCTGATCACATATGGGC-3 5-GACCATTACGTATCAACT-3	498
2	*Brucella ovis*,	5- AGCTGATCACATATGGGC-3 5-CGGCTTCAGCCACCAACG-3	976
3	*Brucella melitensis*	5-ACGCCATCAATCAAGGGC-3 5-AATTCCCGTCCTTGGTGG-3	731
4	*S19* strain	5 -CTCCCGCTAAGAATT-3 5-CTCCATGTTAGCGGCGGT-3	178
5	*RB51* Strain	5-AGCCGATCACTTAAG-3 5-GCCCGAAAGATATGCTTC-3	364
6	Rev1 Strain	5-TGGACCCTTAGCCGTTGGACT-3 5-TCCACGGCAAGTCACGTTAACC-3	211

2.4 PCR

DNA samples were PCR tested for detection of Brucella abortus, Brucella ovis, Brucella melitensis, RB51 strain of B. abortus, S19 strain of B. abortus and Rev1 strain of B. melitensis by AMOS Multiplex PCR method (Bricker et al., 2003). PCR reactions were performed using 13µL of a commercial PCR mix (Accupower PCR preMix, Bioneer, S. Korea), 0.75µL of a 25pM solution of each primer (Table 1), and 1µL of DNA (100 to 500ng per reaction). Parameters used were initial denaturation at 95°C for five minutes, followed by denaturation at 95°C for one minute, annealing at 55.5°C for one minute, extension at 72°C for one minute and a final extension at 72°C for seven minutes. The annealing temperatures and number of cycles for each agent are described, too. PCR products were resolved by electrophoresis in a 1.5 percent agarose gel stained with ethidium bromide. Positive controls included DNA from cultured organisms or infected tissues. Positive and non-template controls (in which the DNA template was replaced by PCR-grade water) were included with all reactions.

2.5 Statistical analysis

Frequencies of positive results were compared between PCR and ELISA tests by the Fisher's exact text, using SPSS software, version 16 (Graphpad Software, Inc., CA, USA).

3. Results

Six out of 76 dams (7.8 percent) were seropositive to the Brucella spp. and six out of 76 aborted fetuses (7.8 percent) showed positive reaction by the PCR tests (Table 2).

Table 2. Frequencies of abortions caused by *Brucella Spp*. detected by PCR (in fetal tissues) and ELISA tests (in dam's sera).

Test	Positive	Negative	Total
ELISA	6 (cows) (7.8%)	70 (cows) (92.2%)	76 (100%)
PCR	6(fetuses) (7.8%)	70(fetuses) (92.2%)	76 (100%)

Of six PCR positive samples, four fetuses (66 percent) showed positive reaction against the Brucella abortus and the two remaining (34 percent) against its vaccine strain, RB51 respectively (Fig.1 & Table 3). Statistical analysis did not show any significant difference between two diagnostic (PCR and ElISA) tests for overall detection of abortions caused by Brucella spp. However, only PCR protocol had the ability to differentiate Brucella strains from each other.

Table 3. Frequencies of abortions caused by *Brucella abortus* and RB51 strains detected by PCR in aborted fetal tissues.

Strain	Positive	Negative
B. abortus	4(66%)	2(34%)
RB51	2(34%)	4(66%)
Total	6(100%)	6(100%)

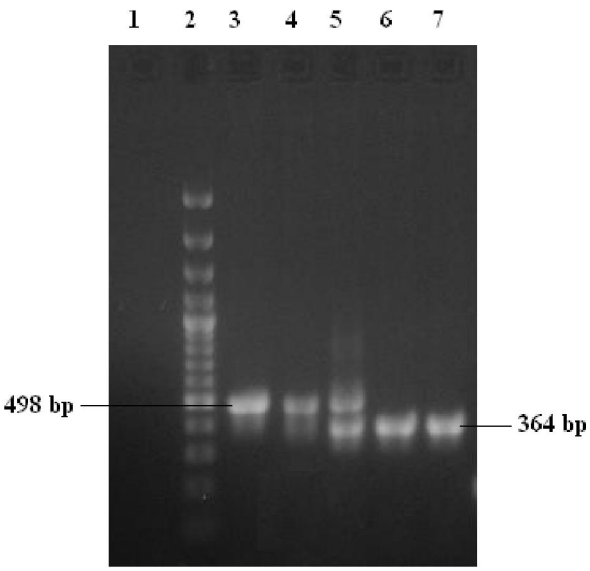

Fig. 1. Representative results of PCR amplification of genomic DNA of *B. abortus* and RB51 in fetal tissues: Lane 1: Non Template Control (NTC); Lane 2: molecular weight marker, Lane 3 & 4: samples from aborted fetuses; Lane 5: Multiplex positive control, upper lane (498 bp) for *B. abortus* and lower lane (364 bp) for RB51 strain; Lane 6 & 7: samples from aborted fetuses.

4. Discussions

Abortions have a highly negative impact on reproductive efficiency, resulting in significant economic losses for the cattle industry (Da Silva et al., 2009). The exact number of abortions due to infectious agents is not known, but in 90 percent of cases in which an etiologic diagnosis is achieved the cause is infectious (Nascimento et al., 2003). Brucellosis once was considered to be the most prevalent reproductive disease of cattle (Youngquist and Threlfall, 2007). Because of its major economic impact on animal health and the risk of human disease, most countries (including Iran) have attempted to provide the resources to eradicate the disease from the domestic animal population. Control programs have employed two principal methods: vaccination of young or mature animals, and the slaughter of infected and exposed animals, usually on the basis of a reaction a serological test (Radostitis et al., 2007). Serology is a standard method for the epidemiological surveillance of brucellosis (Leuenberger et al., 2007 and Köppel et al., 2007). However, cross-reactions between Brucella species and other Gram-negative bacteria are a major problem of the serological assays (Kittelberger et al., 1995, Muñoz et al., 2005). Molecular diagnostic techniques represent an important breakthrough in the diagnostic practice. A number of genus- or species-specific conventional PCR assays using

primers derived from different gene sequences from the Brucella genome, such as 16S rRNA (Herman, 1992) , the 16S-23S intergenic spacer region (Rijpens et al., 1996) omp2 (Leal-Klevezas et al., 1995) and bcsp31(Baily et al.,1992) have been established. These assays were adapted for application to Brucella detection in different clinical specimens. In the majority of studies, PCR proved to be a good means to detect Brucella DNA from clinical specimens (Leal-Klevezas et al., 1995, Keid et al., 2007) while Romero and colleagues found that PCR had lower sensitivity compared to conventional detection methods (Romero et al., 1995). On the other hand Ilhan et al (2007) have emphasized the importance of using more than one type of diagnostic technique for the detection of animals positive for brucellosis, especially for epidemiological purposes (Ilhan et al., 2008). Based on the above-mentioned reasons and for more confidence, we decided to perform two different tests (PCR and ELISA) for diagnosis of abortions caused by Brucella spp. in the Tabriz dairy herds. Our results indicated that ELISA and PCR protocols have equal value for diagnosis of abortions caused by brucella spp. However, it seems that PCR protocol is more reliable than ELISA test because of its ability to determine different strains of Brucella spp. from each to other, particularly about the vaccine strains (RB51), where the positive animals must be slaughtered. In spite of the Iranian Veterinary Organization program for control and eradication of brucellosis, this disease is still a major threat for dairy herds of Tabriz (or Iran) and further investigation is required for eradication of this disease.

In conclusion, we recommend two different tests for etiological diagnosis of bovine abortions. One of these professional tests could be the PCR protocol, which is a very important tool for detection of bacterial strains, particularly in the cases of abortions caused by Brucella spp. Moreover, for the first time in Iran, our results indicated that the vaccine strain of Brucella abortus (RB51) is not fully safe and should be used cautiously in advanced pregnant animals.

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References

1. Anderson, M.L. 2007. Infectious causes of bovine abortion during mid- to late-gestation. Theriogenology. 68, 474-486.
2. Baily GG, Krahn JB, Drasar BS, Stoker NG. 1992. Detection of Brucella melitensis and Brucella abortus by DNA amplification. J Trop Med Hyg, 95. 271-275.
3. Bricker, B.J., Ewalt, D.R., Olsen, S.C. and Jensen, A.E. 2003. Evaluation of the Brucella abortus species-specific polymerase chain reaction assay, an improved version of the Brucella AMOS polymerase chain reaction assay for cattle. Journal of Veterinary Diagnostic Investigation, 15 (4). 374-378.
4. Cortez, A., Castro, A. M. G., Heinemann, M. B., Soares, R. M., Leite, R. C., Scarcelli, E., Genovez, M. E., Alfieri, A. A., Richtzenhain, L. J. 2006.Detecção de ácidos nucléicos de Brucella spp., Leptospira spp., herpesvirus bovino e virus da diarréia viral bovina, em fetos bovinos abortados e em animais mortos no perinatal. Arquivo Brasileiro de Medicina Veterinária e Zootecnia. 58(6). 1226-1228.
5. Da Silva, T.M.A., De Oliveira, R.G., Da Silva Mol, J.P., Xavier, M.N., Da Paixão, T.N., Cortez, A., Heinemann, M.B., Richtzenhain, L.J., Lage, A.P., De Lima Santos, R. 2009. Etiologic diagnosis of bovine infectious abortion by PCR. Cienc. Rural Vol.39 No.9 Santa Maria Dec.
6. Herman L., De R.H.1992. Identification of Brucella spp. by using the polymerase chain reaction. Appl Environ Microbiol, 58. 2099-2101
7. Ilhan, Z., Aksakal, A., Ekin, I.H, Gulhan, T., Solmaz, H., Erdenlig, S. 2008. Comparison of culture and PCR for the detection of Brucella melitensis in blood and lymphoid tissues of serologically positive and negative slaughtered sheep. Lett Appl Microbiol, 46(3). 301-306.
8. Keid, L.B, Soares, R.M., Vasconcellos, S.A., Chiebao, D.P., Megid, J., Salgado, V.R., Richtzenhain, L.J. 2007. A polymerase chain reaction for the detection of Brucella canis in semen of naturally infected dogs. Theriogenology, 67. 1203-1210.
9. Kittelberger, R., Hilbink, F., Hansen, M.F., Ross, G.P., Joyce, M.A., Fenwick, S., Heesemann, J., Wolf-Watz, H., Nielsen, K. 1995. Serological crossreactivity between Brucella abortus and Yersinia enterocolitica 0:9 – II the use of Yersinia outer proteins for the specific detection of Yersinia enterocolitica infections in ruminants. Vet Microbiol, 47. 271-280.
10. Köppel, C., Knopf, L., Ryser, M-P., Miserez, R., Thür, B., Stärk, K.D.C. 2007. Serosurveillance

for selected infectious disease agents in wild boars (Sus scrofa) and outdoor pigs in Switzerland. Eur J Wildl Res, 53. 212-220.
11. Leal-Klevezas, D.S., Lopéz-Merino, A., Martínez-Soriano, J.P. 1995. Molecular detection of Brucella spp.: rapid identification of B. abortus biovar I using PCR. Arch Med Res, 26. 263-267.
12. Leal-Klevezas, D.S., Martínez-Vázquez,I.O., García-Cantu,J., López-Merino,A. and Martínez-Soriano, J.P., 2000. Use of polymerase chain reaction to detect Brucella abortus biovar 1 in infected goats. Veterinary Microbiology. 75(1). 91-97.
13. Leal-Klevezas, D.S., Martínez-Vázquez, I.O., García-Cantu,J., López-Merino, A. & Martínez-Soriano, J.P. 1995. Single-step PCR for detection of Brucella spp. from blood and milk of infected animals. J Clin Microbiol, 33. 3087-3090.
14. Leuenberger R, Boujon P, Thür B, Miserez R, Garin-Bastuji B, Rüfenacht J, Stärk K.D. 2007. Prevalence of classical swine fever, Aujeszky's disease and brucellosis in a population of wild boar in Switzerland. Vet Rec, 160. 362-368.
15. Martínez-Soriano, J.P. 2000. Use of polymerase chain reaction to detect Brucella abortus biovar 1 in infected goats. Veterinary Microbiology, 75(1). 91-97.
16. Muñoz, P.M., Marín, C.M., Monreal, D., González, D., Garin-Bastuji, B., Díaz, R., Mainar-Jaime, R.C., Moriyón, I., Blasco, J.M. 2005. Efficacy of several serological tests and antigens for diagnosis of bovine brucellosis in the presence of false-positive serological results due to Yersinia enterocolitica O: 9. Clin Diagn Lab Immunol, 12. 141-151.

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17. Nascimento,E.F., Santos,R.L. (2nd.Edn.) , 2003. Patologia da reproducao dos animais domesticos. Rio de Janiro: Guanabara Koogan, pp. 137.
18. Noakes, D.E., Parkinson, T.J. & England G.C.W. (9th Edn.), 2009. Arthur's veterinary Reproduction & Obstetrics. Philadelphia, W.B. Saunders Co., pp.483-486.
19. Radostitis, O.M., Gay, C.C., Hinchcliff, K.W. & Constable, P.D., (10th Edn.) 2007. Veterinary medicine. Spain, W.B. Saunders Co., pp. 963-984.
20. Richtzenhain, L.J, Cortez, A., Heinemann,M.B., Soares, R.M., Sakamoto, S.M., Vasconcellos, S.A., Higa, Z.M.M., Scarcelli, E. & Genovez, M.E. 2002. A multiplex PCR for the detection of Brucella spp. and Leptospira spp. DNA from aborted bovine fetuses. Veterinary Microbiology, 87. 139-147.
21. Rijpens N.P, Jannes, G., Van Asbroeck, M., Rossau, R., Herman, L.M. 1996. Direct detection of Brucella spp. in raw milk by PCR and reverse hybridization with 16S-23S rRNA spacer probes. Appl Environ Microbiol, 62. 1683-1688.
22. Romero, C., Pardo, M., Grillo, M.J., Diaz, R., Blasco, J.M., Lopez-Goñi, I. 1995. Evaluation of PCR and indirect enzyme-linked immunosorbent assay on milk samples for diagnosis of brucellosis in dairy cattle. J Clin Microbiol, 33, 3198-3200
23. Youngquist, R.S., & Threlfall, W. R.(2nd. Edn). 2007. Current Therapy in Larg Animal Theriogenology, U.S.A., Saunders Company, ISBN: 10:0-7216-9323-7, pp.390-391.

Talent Management: A Complementary Process for Succession Planning

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Abstract: Nowadays, talent management has become an essential priority for modern organizations, and organization success is directly related to talent that is used. The aim of this paper is to focus on the importance of talent management in organizations, and study its relationship with succession planning. With an overview of journals and books and related reports, documents show that talent management is a complementary, effective process for succession planning. Organizations focus on talent management causes them to successfully attract, retain and develop their essential talents. In other words, talent management is a subsidiary of succession planning, and succession planning is a subsidiary of human resource planning. In spite of a greater need for scientific researchers, organizations must allocate sufficient resources and specific attention to talent management for their survival.
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Keywords: Talent; Talent management; Talent pool; succession planning

1. Introduction

While in the past, manpower faced the problem of shortage occupation, modern organizations are dealing with talent scarcity, and demand for talents is much higher (D'Amato & Herzfeldt, 2008). Hence, talent pool and talent management from countries and organizations have been targeted, and economists have also proven that finding talented people, as one of the world's most valuable products, has been very difficult. Studies show that the concept of talent for any organization is unique (Lutbish & Smith, 2007). Based on experience gained, talent is defined, and through the ability to learn skills that will prove the person comes on complex tasks (Brusman, 2001). In other words, talent can be considered as a complex combination of skills, knowledge, cognitive ability, and potential employees. Based on high-performance and high-ability, talent is defined, and organizations set their priorities based on these two dimensions: some organizations have more emphasis on talent diagnosed in talent management programs, whereas some talent management approaches are associated with identification, tracing and keeping the best individuals. By examining the structure of talent, its strategies can be summarized in the following:

1. Defining the framework of capabilities associated with the organization development for top/middle managers;
2. Deep talent evaluation based on framework of capabilities for existing staff and new recruits; and
3. Identifying vital positions according to organization strategies (Sharma & Bhatnagar, 2009).

Considering the position of talent management in modern organizations, the purpose of this study is to focus on the importance of talent management as complementary to succession planning.

2. Talent management

The definition of talent management is difficult because it is a complicated task that usually is used within the strategy of human resources. Talent management is the process of attracting, transferring, developing, and strategies that are related to those activities in an organization (Rothwell, 2005), and it is a process and opportunity to help management provide for individuals in the organization as individuals with talent (Blass, 2007). Today, talent management as a broader concept is known in order to attract, retain, and develop talents (D'Annunzio-Green, 2008), and it is important for two reasons: first, talent management will ensure that organization can successfully attract and maintain necessary talent. The second reason is talent management should be done in a field that employees are working within (Hughes & Rog, 2008). According to McGee (2007), effective work with talented people is based on talent management strategy that is derived from the organization strategy. Talent management strategy is trying to ensure that existing talented, worthy, and

committed people are contributing in achieving the existing and future needs of organization, and this procedure is called the talent pool (Horvathova, 2009). Talent pool is a group of people ready for responsibilities. People who are placed in a talent pool may appear with various abilities. An approach is that managers are asked to evaluate and select individuals. Another approach is using objective assessment methods such as multilateral evaluation to identify individuals who likely would be useful for future responsibilities (Rothwell, 2006). Developed talent pool is introduced to replace traditional succession planning. In this system, managers don't worry about who helps them. CEOs, regardless of daily work have more time to track skills of pool members—that is, develop future's managers. The size of developed talent pool depends on the number of executive position, and the ability of an organization to develop. In an average organization, there may be one pool, but in large organizations may be two pools- one in the senior management levels and the other in middle management levels. Number of pools often reflects the organizing of organization (Byham, 2001).

Talent management occurs in several forms and sizes. One of the most effective results in this area is the scope of talent management that could be coding and planning strategic priority and vision of talent management approach. Strategic vision will shape the organization in which the talent management system is examined and performed. This vision needs to be understood and be supported from people who run the system; otherwise, the process of talent management can be different people distort or be neutral (Devine & Powell, 2008). Generally two options for strategic management exist. These options include:

1. Aligning People with Roles. It is assumed here that there are acceptable roles, and the purpose is matching people with roles. Four main factors in the performance of people in this area are:

a) Selection, recruitment, appointment and promotion;
b) Learning and development;
c) Succession planning; and
d) Career instructions.

2. Aligning Roles with People. This strategic area based on attracting individuals as fixed and adapted factor in organization tissue. Relevant factors in this area include:

a) Organization design;
b) Role design;
c) Rewards;
d) Work environment; and
e) Working method (Cunningham, 2007).

In another study, it has been identified six strategic visions for shaping the organization's approach to talent management which include:
Competitive vision: this vision is based on this idea that talent management must identify the talents and their demands should be noticed, otherwise these people are kidnapped by competitors. This is a contractive state in organization that there is no formal process for talent management—talent management effectively works as a maintenance strategy. Studies show that professional services firms and companies with competitive sectors, such as banking and financial affairs, use this strategy.

a) **Process vision:** This vision based on process that will optimize the performance and is rooted in the idea that future success is based on good talent. Managing and developing talent is a part of the daily process of organization.
b) **Human resource planning vision:** This vision is similar to process vision, but the orientation of human resources for aligning right people for right jobs at right time and do right things reflected correctly. HR teams often control and monitor the process of talent management. This vision is done often by companies that have experienced rapid growth.
c) **Development vision:** This vision concentrate to abilities or talents, and talent management often rapidly get through the development paths.
d) **Cultural vision:** It includes talent management image as a strong way of thinking, so that vitalizes talent for organizational success.
e) **Change management vision:** Talent management as a stimulus to change is considered, and could be part of strategic HR for organizational change, perhaps due to a change of ownership or a new set of governmental reforms. Talent management can help to change organizational culture, leadership style and experts and managers capabilities (Devine & Powell, 2008).

Furthermore, talent management development to some extent specifies development paths for potential employees (Wilcox, 2005). An important point to formation of talent management is that this formation is different at organizational levels. At the lower level, no strategy, policy or formal developed activity exist. While at a higher level, talent management strategy was formed and it

is specified through the organization strategy. At this level, individual and group talent is identified (Chartered Institute of Personnel and Development, 2006). Studies show that the talent management system is divided into three main areas: talent recruitment, talent maintenance, and talent development. These areas are described below:

a) **Talent recruitment:** This stage includes all issues related to individual with high-level skills: what people does the organization need to invest in? How should the organization plan for recruitment at determined positions? What kind of talent is needed for organizational development? These questions are only a part of the questions in an organization, while drawing a path of attraction and development of its employees, as most valuable investment should be considered.
b) **Talent maintenance:** When an organization was successful in recruitment on desired post, they should know what the next step is. At this step, the appropriate skill sets of individual required to locate with job duties. On the other hand, manpower performance should manage to ensure the organization is a path that has the most productivity by HR.
c) **Talent development:** The final step in this process including issues related to learning and development. In this phase, employees need a transparent and palpable career path. The organization need to more investment on employees, in order to meet future expectations and needs (Tajadin & Muali Taffti, 2009).

Organizations notice to talent management is important, so that they could recruit, maintain and develop their needed talent; or intended to discover talents inside or outside of organizations.

3. Discussions

Succession planning (SP) is one of subsidiaries of HR planning that in which vital positions is specified and it ready employees to qualify for given positions. SP is so named as "Building Bench Strength" (Phillips & Redmon, 2010). Barton and Rouse-Jouns (2007) are summarized the importance of SP in these cases: ensuring to continuance of prepared leaders for key positions; having a systematic process for examining leadership talent; increasing opportunities for talents; participating to organization programs; helping people to identify their career plans within the organization; and developing the strong leadership teams for strategic tasks.

Although there are many models regarding SP, the most common model in this area is provided by Rothwell. Rothwell (2006) suggest nine basic step for effective SP process, which include: identifying managers expectations; drawing a capabilities model; performing multiple assessment; creating performance management system; evaluating talented people; planning a tool for ongoing development; doing development plans; providing bench strength; having individual and organizational accountability; and evaluating the results. With the recognition of SP process, it can be acknowledged that the boundary between talent management and SP is not clear. Like talent management, SP is a complex process that has many layers and levels. Some managers may know talent management equal to SP, and some know it is equal with employment, while others know talent management and leadership development are the same. Lack of connection between SP with talent management can create problems, such as existence of a clear talent management process and an ambiguous SP process. Respectively, talent management can be an effective complementary process for SP or sometimes it could combine SP to leadership process and organization management (Berger & Berger, 2004). Hence, SP is the ultimate goal of talent management. One of the goals of developing management program is creating a talent pool to complete the specific job classes with focusing on individual skills, capabilities, and behaviors. Without developing future managers, it is impossible to creating a SP.

4. Conclusion

Talent management has been major priority for many organizations and success of today's organizations directly linked with used talents. Talent identification and development, which is known as talent management refer to the process by which the organization identifies employees who are capable to play leadership role in future. This approach emphasizes developing talent pools that have high leadership ability. Also, the organization should define a clear vision to talent management and draw a map to integrate technology and processes. On the other hand, organizations should pay attention to all members within organizations for talent management, and put middle managers responsible for recruitment staff. Talent management and SP are dynamic processes, which occur at variable times. SP needs to adapt with other sector of HR management, such as talent management, learning processes, development and performance. Talent management can be an effective, complementary process for SP. Therefore, talent management is a component of SP and organization is seeking to implement SP should

execute talent management in their organization. In other words, these organizations should be able to manage talent supply and demand to achieve optimal performance along organizational goals as a part of SP. Therefore, in today's organization, talent management needs to transform from a support activity to a competitive activity.

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References

1. Barton, Karen V. & Rouse-Jones, Margaret (2007), "Leadership and Succession Planning in Caribbean Libraries," Workshop on Trends and Development in Caribbean Librates, June 25-29, pp. 4-5.
2. Berger, L.A. & Berger, D.R. (2004), "The talent management handbook," New York: McGraw-Hill, p. 2.
3. Blass, E. (2007), "Talent Management: Maximising Talent for Business Performance," Chartered Management Institute/Ashridge Consulting, p. 1.
4. Brusman, Maynard (2001), "Working Resources: Helping Companies Assess, Select, Coach and Retain Emotionally Intelligent People," Newsletter, Vol. IV, No. 4, p. 3, www.workingresources.com.
5. Byham, William (2001), "Taking Your Succession Management Plan into the 21st Century," Development Dimensions International, Inc., pp. 1-6, http://www.ddiworld.com.
6. Chartered Institute of Personnel and Development (2007), "Be Structured in Managing Talent," Development and Learning in Organizations, Vol. 21, No. 3, p. 31.
7. Cunningham, Ian (2007), "Talent Management: Making it Real," Development and Learning in Organizations, Vol. 21, No. 2, pp. 4-5.
8. D'Amato, Alessia & Herzfeldt, Regina (2008), "Learning orientation, organizational commitment and talent retention across generations," Journal of Managerial Psychology, Vol. 23, No. 8, p. 930.
9. D'Annunzio-Green, Norma (2008), "Managing the Talent Management Pipeline," International Journal of Contemporary Hospitality Management, Vol. 20, No. 7, p. 807.
10. Devine, Marion & Powell, Marcus (2008), "Talent Management in the Public Sector," The Ashridge Journal, pp. 3-5, www.ashridge.org.uk/360.
11. Hughes, Julia & Rog, Evelina (2008), "Talent Management: A Strategy for Improving Employee Recruitment, Retention and Engagement within Hospitality Organizations," International Journal of Contemporary Hospitality Management, Vol. 20, No. 7, p. 746.
12. Horvathova, Petra (2009), "Enterprise: Performance and Business Processes, Perspectives of Innovations," Economics & Business, Vol. 3, p. 77, www.pieb.cz.
13. Lutbish, G & Smith, I. (2007), "Talent Management: A Strategic Imperative," The Ashridge Journal. p. 6.
14. Phillips, Caroly & Redmon, Trish (2010), "Succession Planning: A Key Sustainability Strategy," Succession Planning Webinar, Jan. 19, p. 4, www.passitoncenter.org/content/.
15. Rothwell, W. J. (2005), "Effective Succession Planning: Ensuring Leadership Continuity and Building Talent from Within." New York, NY: American Management Association, p. 79.
16. Rothwell, William J. (2006), "Ten Key Steps to Effective Succession Planning," Rothwell & Associates Inc., pp. 4-11, http://www.halogensoftware.com.
17. Sharma, Rakesh & Bhatnagar, Jyotsna (2009), "Talent Management – Competency Development: Key to Global Leadership," Industrial and Commercial Training, Vol. 41, No. 3, p. 120.
18. Tajadin, M. & Moali, M. (2006), "Why Talent Management?" Tadbir Journal, No. 191: 62.
19. Wilcox, I. (2005), "Raising Renaissance Managers," Pharmaceutical Executive, Vol. 25, No. 6, p. 41.

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$CD4^+CD25^+$ regulatory T cells suppress the *M.TB*-specific immune response of T cells

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Abstract: To investigate the negative regulatory effect of macaques $CD4^+CD25^+$ regulatory T cells (Tregs) on anti-*M.TB* immune response of memory T cells in vitro through determining the suppression of Tregs on the purified protein derivative (PPD)-specific proliferation of memory T cells. Peripheral bloods were drawn from 6 male *Rhesus macaques (RH)* adminimistrated by BCG within 3 months, and then $CD4^+CD25^+$ T cells were separated from PBMCs by immunomagnetic beads and labeled with PKH26 red. Cultured the remain $CD4^+CD25^+$ T cells-depleted, CFSE-labeled PBMCs alone or in the presence of purified $CD4^+CD25^+$ T cells to 8th day and then both were stimulated by PPD antigen or purified CD3, CD28 antibodies respectively. The proliferations of $CD4^+$ T cells, $CD8^+$ T cells and Vγ2Vδ2 T cells in PBMCs were analyzed by flow cytometry to determine the dilution of CFSE fluorescence intensity and to exclude $PKH26^+$ cells: the percentage of proliferation was calculated on the number of $CFSE^{dim}$ cells divided by the number of $CFSE^+$ cells. The results showed that PPD droved the proliferations of not only memory $CD4^+$ T cells (p <0.001) and $CD8^+$ T cells (p <0.01), but also Vγ2Vδ2 T cells (p <0.05). Besides suppressing the CD3/CD28 antibody-induced non-specific proliferations of $CD4^+$ T cells (p <0.001), $CD8^+$ T cells (p <0.01) and Vγ2Vδ2 T cells (p <0.05) significantly, $CD4^+CD25^+$ T cells have the function of suppressing the PPD-specific proliferations of $CD4^+$ T cells (p<0.001) and $CD8^+$ T cells (p <0.01), Vγ2Vδ2 T cells (p <0.05). These results suggested that Tregs of macaques have negative regulatory effect on anti-*M.TB* immune response of memory T cells in vitro.

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Key words: Tregs; anti-*M.TB* immune response; non-human primate

1. Introduction

Among infectious diseases, tuberculosis is still one of the major fatal diseases in the world (Huang et al, 2007): there are 8～9 million new cases of TB and 1.5～2.5 million cases of deaths annually in the world (Li et al, 2007). Attenuated BCG has been proved effective in preventing children tuberculosis in developing countries (Soysal et al, 2005). However, it is not very clear of the mechanisms about pathogenesis and anti-*M.TB* immunity, which greatly impedes the improvement of TB prevention and control. Therefore, that elucidating anti-*M.TB* immunity triggered by BCG infection will be very beneficial to find new effective measures to prevent and control *mycobacterium tuberculosis* infection (Huang et al, 2007).

Tregs play key role in the process of maintaining immune balance during infection, tumor and autoimmune diseases by suppressing the activation, proliferation and/or effective function of effective T cells (Li et al, 2007). Our (Gong et al, 2009) and other's studies (Li et al, 2007/2008; Chen et al, 2007; Scott-Browne et al, 2007) recently indicated that Tregs may have the negative regulating function in primate anti-*M.TB* immunity.

Because their biological characteristic are very close to human, non-human primate have been used as precious human infectious disease model. Of most concern, studying the anti-infectious immunity of non-human primates with human infectious disease will be of great significance and importance in reality in preventing and controlling human mycobacterium tuberculosis. In this study, we determined the regulatory function of non-human primate Tregs on effective T cells such as $CD4^+$ T, $CD8^+$ T cells and Vγ2Vδ2 T cells during anti-M.TB immunity *in vitro*.

2. Material and Methods

Animals

6 male RH macaques were provided by Biological Resource Center in the University of Illinois at Chicago (UIC). At beginning of the study, their ages ranged from 3 to 10 years old and body weight from 2.3 to 15.1 kg. All animals were maintained and used in accordance with guidelines of Institutional Animal Care and Use Committee (IACUS) at UIC. Animals were anesthetized with 10 mg/kg ketamine HCl (Fort Dodge Animal Health, Fort Dodge, IA) i.m. before drawing blood samples and being treated. All monkeys were artificially introvenous infected with BCG at a

dose of 10^7 in 0.5 ml volume within 3 months.

Cell isolation

5~ 15 mL of EDTA blood samples were collected from each macaque before and after BCG infection. Then PBMCs were separated from EDTA-anticoagulant peripheral blood using density gradient method. $CD4^+CD25^+$ T Cells were purified by using $CD4^+CD25^+$ Regulatory T Cell Isolation Kit for non-human primate (Miltenyi Biotec). Briefly, $CD4^+$ T cells were purified from PBMCs by depletion of non-$CD4^+$ cells with negative selection. From purified $CD4^+$ T Cells, $CD25^+$ T Cells were isolated by positive selection of $CD4^+CD25^+$ regulatory T cells using $CD25^+$ magnetic microbeads. $V\delta2^+$ T cells were purified by using purified mouse-anti-human Vδ2 (Clone: 15D, Endogen, Rockford, IL) Abs and goat-anti-mouse IgG microbeads (Miltenyi Biotec) with positive selection.

Label Cells with CFSE and PKH26

6×10^6 of $CD4^+CD25^+$ T cells-depleted PBMCs were prepared and labeled with CFSE using the CFSE Cell Proliferation Kit (Invitrogen-Molecular Probes) following the manufacturer's protocol. Briefly, the cells were suspended gently in 1 ml of prewarmed 0.1 % BSA -PBS containing CFSE at a 2.0 μM concentration and then incubated for 15 min at 37°C in dark. 5 volumes of ice-cold culture media were added to the cells and incubated 5 min on ice to quench the staining. Then the cells washed by resuspending the pellet in fresh media for there times.

The purified 1×10^6 of $CD4^+CD25^+$ T cells were also labeled with PKH26 red using the PKH26 red fluorescent cell linker kit (Sigma) following the instructions. Briefly, the cells were suspended in a 2 ml total volume at final concentrations of 2×10^{-6} M PKH26 dye at room temperature for 5 minutes. Stop the staining reaction by adding an equal volume of complete medium. The cells were then washed for total 4 times before being used.

CFSE-based proliferation Assay

For the suppression assay, the CFSE-labeled, $CD4^+CD25^+$ T cells-depleted PBMCs were added at 2×10^5 cells/well to individual wells of Costar round-bottom 96-well plates supplied with 0.2 ml of prewarmed R1640 with 10% FBS, 50 U/ml penicillin and 50 μg/ml streptomycin and stimulated by 15 μg/ml PPD (Colorado Serum Company), or 5 ug/ml purified mouse anti-human CD28 (CD28.2, BD Pharmingen) and CD3 (SP34.2, BD Pharmingen). Meanwhile, PKH26 labeled $CD4^+CD25^+$ T cells were then added to the CFSE-labeled, $CD4^+CD25^+$ T cells-depleted PBMCs at 2×10^5 /well in the absence or presence of PPD, CD28 and CD3.

After cultured for 7 days in the CO_2 incubator at 37℃, 95 % humidity and 5 % CO_2, the cells were collected and then stained with surface Abs specific for Vγ2 (7A5, Endogen) in each tube at 4℃ for 20 min. After washed 3 times with 5% FBS-PBS, cells in each tube were added 5 uL Goat anti-mouse IgG-APC (Biolegend) at 4℃ for 20 min in dark. After washed 3 times, add surface Abs specific for CD4-Pacific Blue (OKT-4, eBioscience), CD8-PECy7 (DK25, DakoCytomation) in each tube at 4℃ for 20 min in dark. Proliferation response was analyzed by flow cytometry to determine CFSE signal intensity and to exclude $PKH26^+$ cells: the percentage of proliferation was calculated on the number of $CFSE^{dim}$ cells divided by the number of $CFSE^+$ cells.

Statistical analysis

Student *t* test was exploited to determine the differences between groups in vitro. $p<0.05$ was considered significant (GraphPad, San Diego, CA, USA).

3. Results

PPD induced antigen-specific proliferation of memory $CD4^+$ T cells, $CD8^+$ T cells and Vγ2Vδ2 T cells

To determine whether Tregs had the function of suppressing the anti-*M.TB* immunity induced by effective T cells such as $CD4^+$ T cells, $CD8^+$ T cells and Vγ2Vδ2 T cells, we use PPD as antigen-specific stimulation to induce the proliferation of $CD4^+$ T cells, $CD8^+$ T cells and Vγ2Vδ2 T cells in the PBLs of *RH macaques* that recently infected with BCG since protein antigen can be recognized directly by memory $CD4^+$ T cells, $CD8^+$ T cells and Vγ2Vδ2 T cells (Gong et al, 2009; Li et al, 2008).

We found that PPD induced the proliferation of not only $CD4^+$ T cells ($p<0.001$) and $CD8^+$ T cells ($p<0.01$), but also Vγ2Vδ2 T cells ($p<0.05$) of BCG-infected *macaques* (Figure 1, 2), excepting BCG-naive *macaques* (data not show). Our results verify that *macaque* memory Vγ2Vδ2 T cells recognized directly protein antigen, which is in accordance with results in human study (Li et al, 2008).

Tregs suppressed the PPD-specific proliferation of memory $CD4^+$ T cells, $CD8^+$ T cells and Vγ2Vδ2 T cells

To further determine whether Tregs had the function of suppressing the anti-*M.TB* immunity induced by effective T cells such as $CD4^+$ T cells, $CD8^+$ T cells and Vγ2Vδ2 T cells, CFSE-based proliferation assay was exploited under the situation that anti-*M.TB* immunity of effective T cells can be

driven by PPD that results have been shown as above.

We found that Tregs have the function of suppressing the PPD-specific induced activation/proliferation of $CD4^+$ T cells, $CD8^+$ T cells and Vγ2Vδ2 T cells（p<0.001~0.05）, as well as the CD3/CD28 antibody-induced antigen non-specific proliferation of those cells *in vitro* (p<0.001 individually) (Figure 1, 2).

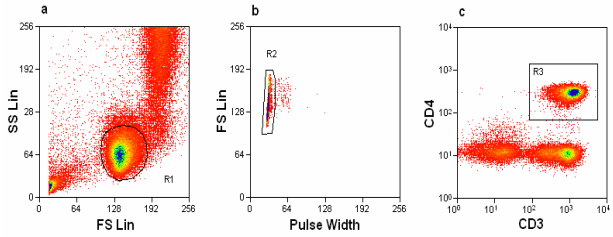

Figure 1. Taking $CD3^+CD4^+$ T cells for example to show the gating strategy. Lymphocytes were gated based on forward and side scatters, and pulse width, and at least 50 000 gated events were analyzed (a→b); Further special gates and quadrants were determined based on nonstaining, isotype control Ab for background staining and specific Ab staining (c). R3 area in figure c represents $CD3^+CD4^+$ T cells gated from R2 area in figure b that is gated from R1 area in figure a.

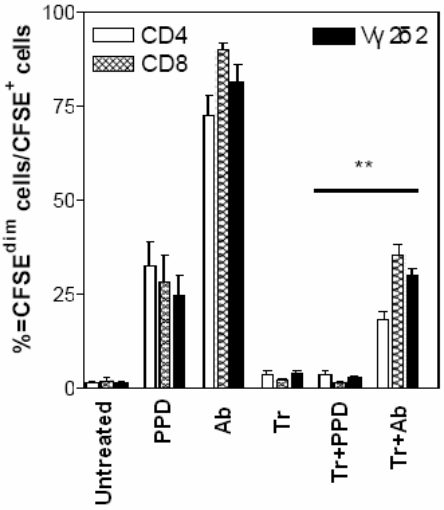

Figure 2. The percentage of suppression of $CD4^+CD25^+$ Tregs on $CD4^+$ T cells, $CD8^+$ T cells and Vγ2Vδ2 T cells in vitro are shown as the mean values of triplicate measurements ±SEM (n=6): ** PPD vs Tr+PPD: CD4, p<0.001; CD8, p<0.01; Vγ2Vδ2, p<0.05. Ab vs Tr+Ab: CD4, CD8 and Vγ2Vδ2, p <0.001.

4. Discussion

In the present study, we conducted a sensitive, versatile CFSE-based assay for detecting the suppressing function of *macaque* Tregs *in vitro* (Gong et al, 2009; Li et al, 2008). By flow cytometric analysis of CFSE-based assay, we found the powerful suppressing functions of Tregs on Vγ2Vδ2 T cells, as well as $CD4^+$, $CD8^+$ T cells in BCG infected *macaque*. Tregs did suppress not only the PPD-specific proliferation of $CD4^+$ T cells which play most important roles in anti-*M.TB* immunity, but also that of $CD8^+$ T cells and Vγ2Vδ2 T cells. Furthermore, Tregs suppressed not only PPD antigen-specific, but also CD3/CD28 antibody-induced non-specific proliferation of conventional $CD4^+$, $CD8^+$ T cells, and Vγ2Vδ2 T cells, which suggested Tregs may control anti-*mycobacterium* immunity that mediated not only by conventional $CD4^+$, $CD8^+$ T cells, but also by Vγ2Vδ2 T cells during anti-*M.TB* immunity. These evidences demonstrated that *macaque* Tregs have versatile regulatory functions on a pool of effector cells including $CD4^+$, $CD8^+$ T cells (Chen et al, 2009) and Vγ2Vδ2 T cells (Gong et al, 2009; Li et al, 2008) which contribute collectively to the extensive ongoing anti-mycobacterium immunity.

Our results provide the first-hand evidence about *macaque* Tregs controlling anti-*M.TB* immunity induced by effective memory T cells, which will further promote the application of *macaques* in the study of human mycobacterium-infected diseases. What's more, the results of this study open up the possibilities to exploit Tregs as potential tool for immunotherapy during infectious or other diseases. For example, pre-activated Tregs may be considered to be used in the future treating acute tuberculosis with brain and pulmonary lesion caused by overactive effective memory T cells which contribute to excessive immune response to result in cerebrocortical necrosis and pulmonary cavity.

In conclusion, our results demonstrated that *macaque* Tregs had the function of suppressing the anti-*M.TB* immunity induced by effective T cells such as $CD4^+$ T cells, $CD8^+$ T cells and Vγ2Vδ2 T cells which is assistant with other studies (Shen et al, 2002; Barboza et al, 2007; Boettler et al, 2005; Cavassani et al, 2006), and will further promote the clinical application of Tregs in controlling and treating intractable infectious diseases.

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References

1. Huang D, Qiu L, Wang R, et al. Immune gene networks of mycobacterial vaccine-elicited cellular responses and immunity. J Infect Dis, 2007;195(1):55-69.
2. Li L, Lao SH, Wu CY. Increased frequency of CD4(+)CD25(high) Treg cells inhibit BCG-specific induction of IFN-gamma by CD4(+) T cells from TB patients. Tuberculosis (Edinb), 2007;87(6):526-34.
3. Soysal A, Millington KA, Bakir M, et al. Effect of BCG vaccination on risk of Mycobacterium tuberculosis infection in children with household tuberculosis contact: a prospective community-based study. Lancet, 2005;366(9495):1443-51.
4. Gong G, Shao L, Wang Y, et al. Phosphoantigen-activated V gamma 2V delta 2 T cells antagonize IL-2-induced $CD4^+CD25^+Foxp3^+$ T regulatory cells in mycobacterial infection.Blood, 2009;113(4):837-45.
5. Chen X, Zhou B, Li M, et al. CD4(+)CD25(+)FoxP3(+) regulatory T cells suppress Mycobacterium tuberculosis immunity in patients with active disease. Clin Immunol, 2007;123(1):50-9.
6. Scott-Browne JP, Shafiani S, Tucker-Heard G, et al. Expansion and function of Foxp3-expressing T regulatory cells during tuberculosis. J Exp Med, 2007;204(9):2159-69.
7. Li L, Wu C. $CD4^+CD25^+$ Treg cells inhibit human memory {gamma}{delta}T cells to produce IFN-{gamma} in response to M. tuberculosis antigen ESAT-6. Blood, 2008;111(12):5629-36.
8. Chen CY, Huang D, Wang RC, et al. A critical role for CD8 T cells in a nonhuman primate model of tuberculosis. PLoS Pathog, 2009;5(4):e1000392.
9. Shen Y, Zhou D, Qiu L, et al. Adaptive immune response of Vgamma2Vdelta2+ T cells during mycobacterial infections. Science, 2002;295(5563):2255-8.
10. Barboza L, Salmen S, Goncalves L, et al. Antigen-induced regulatory T cells in HBV chronically infected patients. Virology, 2007;368(1):41-9.
11. Boettler T, Spangenberg HC, Neumann-Haefelin C, et al. T cells with a CD4+CD25+ regulatory phenotype suppress in vitro proliferation of virus-specific CD8+ T cells during chronic hepatitis C virus infection. J Virol, 2005;79(12):7860-7.
12. Cavassani KA, Campanelli AP, Moreira AP, et al. Systemic and local characterization of regulatory T cells in a chronic fungal infection in humans. J Immunol, 2006;177(9):5811-8.

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Assessment of clinical skills of midwives who graduated from Mashhad school of nursing and midwifery who are employed in hospitals and health centers

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Abstract: Appropriate midwifery skills can prevent deaths and help avoid complications of childbirth. Quality of midwifery training significantly affects clinical skills. Graduate students of midwifery should gain a minimum level of clinical skills to adequately perform their duties. Assessing the graduates' clinical skills can be helpful for evaluating the curriculum. The objective of this research was to determine clinical skills of graduated midwives from Mashhad school of nursing and midwifery who are employed in hospitals and health centers. Present research is a cross-sectional study. Statistical society of this research is a group of 50 recent graduated midwives who have been employed in the health centers of Mashhad from three month up to three years. Fifty heads of health centers were also selected. All of the heads had at least six months of job experience. After following the legal processes and explaining the research objectives and method of filling the questionnaire, the midwives and the heads were asked to complete the questionnaires. Another questionnaire is "assessment of midwives by heads," which is extracted from the other questionnaire. Results of the present research illustrate that more than 95 percent of the essential skills for a midwife are obtained during education. More than 90 percent of the graduated midwives who work in maternity and obstetrics and gynecology and 85.5 percent of midwives in healthcare assessed their knowledge and skills adequate for their job in low-risk situations. But, in high-risk situations, 76.1 percent of graduated midwives who work in the maternity and 77.4 percent who work in healthcare assessed their knowledge and skills satisfactory for doing their duties. From a statistical point of view, self-assessment of the skills for handling low-risk situations is significantly higher than the numbers related to the high-risk situations ($P < 0.05$). The Pearson product moment test illustrated a positive correlation between the acquired skills in the curriculum and increasing self confidence among the graduates ($P < 0.05$). Seventy-one percent of the authorities in midwifery who are employed in the maternity and obstetrics and gynecology and 75 percent of authorities in the health centers assessed the graduates skillful enough to handle low-risk situations. Results of the present research illustrate that the graduates assessed themselves significantly more skillful compared to the authorities' assessment ($P < 0.01$).
[Kobra Mirzakhani, Nahid Jahani Shoorab, Nahid Golmakani, Mahin Tafazoli, Saeed Ebrahimzadeh. **Assessment of clinical skills of midwives who graduated from Mashhad school of nursing and midwifery who are employed in hospitals and health centers.** Life Science Journal. 2011;8(4):482-489] (ISSN:1097-8135). http://www.lifesciencesite.com

Keywords: Assessment; Clinical skills; Graduates of the midwifery

1. Introduction

Maternal death rate is equal to 370/100,000 (Kruske, 2006). In all, over the world, 500,000 women die each year from obstetric complications (Harvey, 2007). Maternal mortality is a catastrophic event and affects the lives of the other family members. Because of these deaths of half a million mothers at least one million children are deprived of having a mother. For each maternal death, at least 15 women suffer complications and chronic deficiencies till end of their lives. Annually 1.8 million neonatal cases suffer from asphyxiation, convulsion, cerebral palsy and mental disorders till end of their lives because of prolonged labor. The World Health Organization (WHO) has declared that death due to asphyxia and injuries during the childbirth are reason for one third of neonatal deaths (Modarres, 2004). Several investigations haves shown that enhancing midwifery care can prevent many neonatal deaths (Harvey, 2004). Fifty-nine percent of the maternal occurs happens in Asian countries.

The rate of maternal mortality in Iran is 37/100,000 and the rate of infant mortality is equal to 16-20 in 1000 births (Modarres, 2004). Previous studies illustrated that appropriate clinical skills and adequate midwifery care during and after labor can prevent more than 75 percent of these mortalities. The World Health Organization (WHO) definition for midwife is: "midwife is a person who is trained for doing necessary clinical skills for handling a normal pregnancy, normal delivery, diagnosis and referring the un-normal cases" (Harvey, 2004).

Midwives need to acquire sufficient skills in family planning, primary care, and neonatal resuscitation (Who, 2001). Midwives play an important role in training and counseling women and families to improve the health level for the families and society. Midwives have a responsibility to train families for prenatal issues, parenting skills, women's health, sexual issues, and infant's cares. Quality of midwifery education affects performance of these duties significantly. Graduates in this field require a minimum level of clinical and professional skills in midwifery to meet the expected goals (Kruske, 2006). Unfortunately, many studies have shown that the quality of midwives' activities is not desirable. Farrokhi, et al. (2007), reported that the quality of provided care by midwives is acceptable only in 55.8 percent of cases (Farrokhi, 2008). Abedzadeh, et al. (2000), mentioned that only 29 percent of midwives do a reasonable job of breast examination (Abedzadeh, 2009).

Curricula should prepare the midwives for performing professional activities in the changing health system for the future (Fleming, 2005). To reach this goal, cooperation, consultation, and comprehensive participation of scholars in midwifery education planning as well as various points of views of the students and university lecturers is essential. By reviewing the midwifery education and obtaining feedback from midwifery training, midwifery courses could be revised. To achieve appropriate changes in the curriculum of midwifery, requirements and essential changes in various parts should be identified. Planning should be done based on these factors. The curriculum development process can be described and identified based on four main components:

1. Knowing the necessity of dynamic planning and choosing the appropriate pattern
2. Improving the details of the curriculum such as objectives and content, learning experiences, methods, and resources
3. Implementation of developed solution
4. Evaluation of previous programs and current programs

One of the components of identifying the curriculum process is evaluation of the performed program (Salehi, 2004). An appropriate method of evaluation is self-assessment (Seyf, 2005). One of the best methods to determine clinical skill and knowledge is self-assessment. Every midwife is an appropriate resource for data collection about himself/herself. Performing the investigation at the beginning of working after educating is important because the midwife is facing problems and requirements and this helps him/her to assess his/her knowledge for doing his/her duties (Nasirian, 2006; Salehi, 2003). Evaluating curriculum for assessing methods of training and course content is essential. Required data about quality of the methods and course content could be obtained from students and graduates (Wentling, 1997). On the other hand, assessment by authorities from a different point of view is a significant component of program evaluation (Salehi, 2003).

By reviewing studies conducted in Iran, a lack of data about this issue can be seen. According to the importance of considering the educational system of midwifery, this study has been conducted for the assessment of clinical skills of graduated midwives from Mashhad school of nursing and midwifery who are employed in hospitals and health centers.

2. Material and Methods

Present research is a cross-sectional study. Statistical society of this research is a group of recent graduated midwives in the last three years including 50 persons, who are employed in private and charity hospitals, maternities, and health centers of Mashhad for a period between three months up to three years. Also a group including 50 persons from authorities of the health centers was selected. All these people had at least six months of job experience. Due to limited statistical society in this research, the sampling method was census, while sample and society are assumed to be the same.

After following the legal processes and explaining the research objectives and method of filling the questionnaire to the midwives and the heads of the departments, they were asked to complete the questionnaires. Meanwhile, all the research samples are ensured that the information will remain confidential.

The applied tool in this study for assessment of the graduated midwives included two different parts:

1- "An instrument for self-assessment of midwife," that is a questionnaire for determining level of capabilities and clinical skills of midwives. This questionnaire contains essential components that should exist in midwifery training courses for high-quality care of women and family to train a competent midwife. More than 40 midwifery skills that result in a decrease in infant mortality and morbidity exist in this questionnaire. This questionnaire was prepared by International Confederation of Midwifery (ICM 2006) by Delphi method and it is approved by the World Health Organization (WHO). Several meetings have been

held by representatives of member states (86 countries) and non-members to provide this questionnaire.

Validity of instruments has been evaluated by members of the International Confederation of Midwifery (ICM) and its reliability has been tested by conducting a study in Cambodia and Mongolia. It has been shown that this instrument has high correlation with the observation method and it has been approved. The questionnaire contains three sections:

a) Demographic characteristics of the graduates including age, job experience, job location, type of degree, and some other components.

b) Self-assessment instrument of midwife in maternity.

c) Self-assessment instrument of midwife in health center.

There are six options for each question in the questionnaire. Four options are related to learning or not learning of a specific skill. Two other options are related to having self-confidence for doing that specific skill.

2- Another questionnaire is "assessment of midwives by heads of the departments." This questionnaire is extracted from the first questionnaire and the person in charge does the scoring for the clinical skills of the midwives based on the Likert scale.

Although validity and reliability of the applied tools were tested out of Iran, these tools were translated to Persian by the researcher and were approved by members of the Faculty of Nursing and Midwifery of Mashhad University from face validity and content point of view. To determine the reliability of the self-assessment tools of the midwives and assessment tools of midwives by heads of the departments, re-test and determining correlation with Chronbach's Alpha were used. After extracting and coding the data, statistical analysis was performed by SPSS software.

3. Results

Average of the age among graduates of the midwifery in this research was 24 years and average GPA was 16.5. According to results of present study, 91.2 percent of the recent graduates of midwifery who are employed in maternities and obstetrics and gynecology departments of hospitals reported that these skills were included in their curricula. They reported having sufficient skills after graduation to handle the following: low-risk pregnancies and deliveries, record patient history, perform venipuncture, bladder catheterization, estimate gestational age, abdominal and vaginal examinations, labor management process, episiotomy, perianal rupture, admissions and primary neonatal care, training and effective assistance to start breastfeeding for mothers.

A various percentage of graduates employed in maternities and obstetrics and gynecology departments of hospitals reported that they had enough training during their education to handle the following skills. Numbers in the parenthesis show the percentage of graduates: CDP (92.9 percent), diagnosis of FHR (83.3 percent), handling FHR (63.6 percent), diagnosis of abnormal presentations (60 percent), breech delivery (54.5 percent), management of labor with umbilical cord prolapse (75 percent), diagnosis and management of level four of obstetric bleeding(84 percent), management of pre-eclampsia (76.9 percent), diagnosis and management of eclampsia (62 percent), diagnosis of emergencies in midwifery (64.3 percent). The average percentage of those graduates who believe they are able to handle high-risk deliveries and above-mentioned skills is equal to 76.1 percent. There is another group of graduates who believe that although they had been trained in mentioned skills, they were not able to perform them after graduation.

Numbers in parenthesis show the percentage of the second group: management of FHR (36.4 percent), diagnosis of abnormal presentations (40 percent), management of breech delivery (36.4 percent), management of pre-eclampsia (23.1 percent), diagnosis and management of eclampsia (36 percent) and diagnosis of midwifery emergencies (35.7 percent). Statistically, self-assessment of skills by graduates of midwifery for management of low-risk pregnancies and deliveries is considerably higher than management of high-risk pregnancies and deliveries ($P< 0.05$).

Another part of this study illustrate that the percentages of midwifery graduates of t assessed their training sufficient for management of the following skills in low-risk situations: prescribing drugs and supplements to pregnant women and training of method of application of these drugs and supplements (90 percent), training for contraception (100 percent), prescribing supplements to neonatal and instruct method of application of these supplement to mothers (70 percent), appropriate and effective control of growth of children under 5 years of age (90 percent), training for children's nutrition, training for vaccination program, apply and interpret routine tests in pregnancy and breast examination (100 percent), pelvic examination (90 percent), performing and interpreting Pap smear (60 percent), and IUD insertion (50 percent). On average, 85.5 percent of graduates assessed themselves knowledgeable and skillful enough in the above skills.

Table 1: Self-assessment of graduates of midwifery from Mashhad universities about pregnancy and high-risk deliveries

Self assessment of clinical skills / Clinical skills	1)This skill was taught during the education and after graduation I was enough skillful to perform it	2) This skill was taught during the education and after graduation I was not enough skillful to perform it	3)This skill was not taught during the education and I learned it in training courses after graduation during employment	4) I never learned this skill	Total
Diagnosis of CPD	92.9%	7.1%			100%
Diagnosis of FHR	83.3%	16.6%			100%
Managing FHR	63.6%	36.4%			100%
Diagnosis of abnormal presentations	60%	40%			100%
Managing breech delivery	54.5%	36.4%	9.1%		100%
Managing delivery with cord prolapse	75%	16.7%	8.3%		100%
Managing long third level	81.8%	18.2%			100%
Diagnosis of bleeding in fourth level of delivery	83.3%	16.7%			100%
Managing bleeding in fourth level of delivery	84/6%	15/4%			100%
Diagnosis of preeclampsia and eclampsia	85.7%	14.3%			100%
Managing preeclampsia and eclampsia	76.9%	23.1%			100%
Diagnosis of eclampsia attackts	61.5%	38.5%			100%
Managing the eclampsia attackts	64.3%	35.7%			100%
Diagnosis of the emergency cases	64.3%	35.7%			100%
Managing the emergency cases	76.5%	23.5%			100%
Neonatal resuscitation by bag and mask	85.7%	14.3%			100%
Diagnosis of un normal cases in neonatals	100%				100%

Table 2: Assessment of authorities and heads of the departments about clinical skills for managing high-risk pregnancies and deliveries

Assessment of Clinical skills / Clinical Skill	Not acceptable	Lower than expected level	Border line	At expected level	Higher than expected level	Total
Diagnosis of CPD	%7.16		%7.16	%7.66		%100
Diagnosis of FHR	%7.16	%7.16		%7.66		%100
Diagnosis of abnormal presentations	%7.16		%7.16	%7.66		%100
Managing the breech delivery	%100					%100
Managing the long third level	%100		%7.16	%7.66		%100
Diagnosis of bleeding in fourth level of delivery	%7.16		%1.9	%7.72		%100
Managing bleeding in fourth level of delivery	%2.18		%1.9	%6.63		%100
Diagnosis of preeclampsia and eclampsia	%3.27	%3.8		%7.66		%100
Managing the preeclampsia and eclampsia	8.3%	%3.8		%3.88		%100
Diagnosis of eclampsia attack	%3.8	%3.8	%3.8	%75		%100
Managing the eclampsia attack	%16.7	%3.8	%3.8	%7.66		%100

Diagnosis of emergency cases		%7.16		%3.83		%100
Managing emergency cases	%16.7			%3.83		%100
Neonatal resuscitation by bag and mask	%8.3	%7.16	%3.8	%7.66		%100
Diagnosis of un normal cases in neonatals	%33.3		%7.16	%50		%100

Also on average, 77.4 percent of the graduates assessed themselves skillful enough and assessed the courses during their education enough to manage the following skills in high-risk conditions: evaluating and training the risk factors in pregnant women (90.9 percent), diagnosis of risky cases in pregnancy (90 percent), diagnosis and management of complications of postpartum (100 percent), consultation and diagnosis of risk factors in neonatal (66.7 percent), diagnosis and management of current diseases among neonatal (88.9 percent), effective training for mothers who are not capable of breastfeeding (84 percent), consultation with mothers who suffer from hepatitis (11.1 percent), diagnosis and treatment of gynecologic infections and management of abnormal breast cases (90 percent).

Statistically, self-assessment of skills for management of low-risk conditions is significantly higher than high-risk conditions by midwifery graduates ($P<0.05$). Extracted results from present research show that the average of self-assessment by graduates about self-confidence for performing clinical skills of midwifery is as follow: 90.2 percent of graduates of midwifery who are employed in maternities and surgical wards in the obstetrics and gynecology departments of hospitals assessed their self-confidence adequate for management of low-risk conditions for reception of patient and 74 percent of them assessed their self-confidence sufficient for management of high-risk.

Table 3: Level of self-confidence for graduates of midwifery from universities and colleges in Mashhad about situations and emergencies. Statistically, there is a significant difference between self-confidence of the graduates in these two situation ($P<0.05$).

Pearson test has shown a positive correlation between acquiring the skills during the education and self-confidence of the graduates for management the situations ($P< 0.05$).

Table 3: Level of self-confidence for graduates of midwifery from universities and colleges in Mashhad about situations and emergencies

Self-assessment of Clinical skills / Clinical skills	I have enough self confidence for doing this skill	I don't have enough self confidence for doing this skill	total
Diagnosis of CDP	%7.91	%3.7	%100
Diagnosis of FHR	%70	%30	%100
Managing FHR	%7.72	%3.27	%100
Diagnosis of abnormal presentation	%3.83	%7.16	%100
Managing breech delivery	%70	%30	%100
Managing delivery with cord prolapsed	%8.77	%2.22	%100
Managing third long level	%8.88	%2.81	%100
Diagnosis of bleeding in fourth level of delivery	%100		%100
Diagnosis of preeclampsia and eclampsia	%100		%100
Managing preeclampsia and eclampsia	%100	%7.7	%100
Diagnosis of eclampsia attack	%3.92	%25	%100
Managing eclampsia attack	%75	%2.18	%100
Diagnosis and managing emergency cases	%8.81	%3.33	%100
Neonatal resuscitation by bag and mask	%7.66	%7.16	%100
Diagnosis of un normal cases in neonatals	%3.83		%100

Table 4: Comparison between graduates of midwifery's clinical skills for managing high-risk and low-risk pregnancies and deliveries from heads of departments' and authorities' point of view, (result for T-test is p=0.04).

Number of skills / Clinical skills At expected level Or higher than that	Number	Average	Deviation
Managing low-risk situations	21	%4.71	2.27
Managing high-risk situations	16	%61	3.30
Total	37		

Results of the present study illustrate that by average 71 percent of the heads of the departments assessed the performance of the graduates higher than the expected level for the following areas: management of low-risk pregnancies and labors, recording patient history, venipuncture, bladder catheterization, estimation of gestational age, abdominal and vaginal examinations, labor management process, episiotomy, perianal rupture, admissions and primary neonatal care, training and effective assistance to start breastfeeding for mothers. Meanwhile, an average of 61 percent of heads of the departments assessed performance of the graduates equal or higher than expected level for management of high-risk pregnancies and labors. Somehow, for diagnosis and management of FHR (33.4 percent), management of breech delivery, management of cord prolapsed (100 percent), diagnosis and management of fourth level of delivery (27.3 percent), diagnosis and management of preeclampsia and eclampsia (33.3 percent) and neonatal resuscitation (25 percent), assessed the capability of the midwives lower than expected level.

Table 5: Comparison between self-assessment of graduates for managing high-risk and low-risk pregnancies and deliveries (T-test result is p=0.027)

Number of skills / Acquired Clinical skills at time of graduation	Number	Average	Deviation
low-risk situations	21	%2.91	8.8
high-risk situations	16	%1.76	3.6
Total	37		

Table 6: Comparison between self-confidence of the graduates for managing high-risk and low-risk pregnancies and deliveries (T-test result is p=0.02).

Numbers / Self confidence of Graduates for	Number	Average	Deviation
Managing low-risk situations	21	2.90	8.8
Managing high-risk situations	16	5.74	3.9
Total	37		

According to the extracted results from this research, an average of 75 percent of the authorities and heads of the departments assessed the capabilities of the midwives for low-risk situations at expected level or higher than that, such as prescribing drugs and supplements to pregnant women and training of method of application of these drugs and supplements and training for contraception (88.9 percent), prescribing supplements to neonatal and train method of application of these supplement to mothers (55.6 percent), appropriate and effective control of growth of children under 5 years of age (44.5 percent), training for children's nutrition (55.6 percent), training for vaccination program (33.3 percent), apply and interpret routine tests in pregnancy (77.8 percent), breast examination (77.8 percent), pelvic examination (88.9 percent), performing and interpreting Pap smear (71 percent) and IUD insertion (88.9 percent). On the other hand, an average of 73 of the authorities and heads of the departments assessed the knowledge and capabilities of the graduates at expected level or higher than that for managing high-risk situations, such as diagnosis of risky cases in pregnancy (77.7 percent), diagnosis and management of complications of postpartum (77.8 percent), consultation and diagnosis of risk factors in neonatal (75 percent), diagnosis and management of current diseases among neonatal (33.3 percent), effective training for mothers who are not capable of breastfeeding (53.4 percent), diagnosis and treatment of gynecologic infections and management of abnormal breast cases (80 percent).

4. Discussions

Extracted results from the present study illustrate that nearly 95 percent of vital skills for midwifery exist in curriculum and students received enough training. 91.2 percent of the graduated midwives who work in the maternity and department of obstetrics and gynecology and 85.5 percent of the employed midwives in healthcare assessed their knowledge and skills adequate for their job in low-risk situations. But, in high-risk situations, 76.1 percent of the graduated midwives who work in maternity and 77.4 percent of the employed midwives in healthcare assessed their knowledge and skills satisfactory for performing their duties. From a statistical point of view, self-assessment of the skills for handling low-risk situations is significantly higher than the numbers related to high-risk situations (P <

0.05). According to a similar investigation, conducted by Kruske et.al (2006), 81 percent of the midwives for performing breech delivery, 91 percent for managing delivery with prolapsed cord, 89 percent for neonatal resuscitation, 56 percent for managing eclampsia, and 93 percent for managing sepsis, assessed their skills lower than expected level and from a statistical point of view assessed their skills for managing low-risk deliveries higher than the high-risk situations (Kruske, 2006). Also Harvey et.al (2004) reported only 48.2 percent of the midwives have enough skills and knowledge for managing bleeding, eclampsia, difficult deliveries, infection, and abortion complications (Harvey, 2004)

To fortify clinical skills in abnormal situations, opportunities should be created for acquiring clinical skills and adequate supervision for students' practicing. To increase clinical skills for handling high-risk situations, training courses should be based on acquiring qualifications, using the training manual, using the check lists and Loog book, including minimum clinical skills (Kruske, 2006). Other research has shown that although more than 90 percent of the courses are based on required lessons, students do not have enough skill to manage uncommon cases such as breech delivery, using forceps and neonatal resuscitation. Also, for training the uncommon cases that have less opportunity to be taught in the clinical environment, Molaje and audio and visual equipment such as films could be utilized to increase the self-confidence of the graduates during the study period (Ehsanpour, 2006). Self-confidence of 90.2 percent of midwifery graduates who are employed in the maternity and department of obstetrics and gynecology in low-risk situations and self-confidence of 74 percent of them was enough to handle high-risk situations. There is a significant difference from a statistical point of view (p=0.05). Self-confidence of 79 percent of graduates who are employed in health centers and 73 percent of them was enough for managing low-risk and high-risk situations, respectively.

In high-risk situations, 61 percent of the authorities in the maternities and 73 percent of the authorities in the health centers assessed the graduates skillful enough to handle the situations. From a statistical point of view, authorities in the maternities and departments of obstetrics and gynecology assessed the skills for handling low-risk situations significantly higher than high-risk situations ($P< 0.05$). Also, results of the present research illustrate that the graduates assessed themselves significantly more skillful in comparison to how the authorities assessed them ($P < 0.01$). In a similar study with an objective related to self-assessment of graduates of nursery about their performance and from heads of departments' point of view, similar results were extracted. To remove the gap between the training courses and practical clinical skills a common planning by training group and clinical group is suggested (Kruske, 2006; Harvey, 2007). The association of American nursing universities applied preceptorship as an effective tool for decreasing the gap between the training courses and practical skills (Udis, 2006). Investigations about applying a preceptorship method in nursery and midwifery training programs found it could be useful for increasing the self-confidence, performance, and clinical skills of the graduates (Allrich, 2001).

Also, using a mentor in training courses for midwifery clinical skills and creating an appropriate environment could be effective for increasing the motivation in learning and elevating the performance of the midwifery graduates (Carlisle, 2009). To fortify clinical skills, especially in high-risk situations, and increase clinical skills for handling high-risk situations, training courses should be based on acquiring qualifications, using a training manual, check lists, and the Loog book, including minimum clinical skills. For training in uncommon cases where there are fewer opportunities to learn in a clinical environment, Molaje and audio and visual equipment such as films could be utilized to increase graduates' self-confidence. To increase the points of evaluation of the heads of the departments and authorities, collaborations and common planning should be arranged between the training group and clinical group. This will result in training more skillful midwives and will elevate the level of health in infants and mothers.

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References

1. Abedzadeh, M., et al. Consideration of performance of midwives who are employed in health centers of Tehran in case of breast examination during 1996. Feiz Journal, No. 15, Fall 2009: 49-55.

2. Allrich, R. P. Investigation of learning within a nursing preceptorship clinical experience: A natural inquiry. Department of Nursing and Midwifery. Ph.D. thesis. The University of Texas at Austin. December 2001. ProQuest.
3. Carlisle, C., et al. Practice-based learning: The role of practice education facilitators in supporting mentors. J.Nedt 2009.02.01. Elsevier Ltd.
4. Ehsanpour. Acquiring to minimum vital skills from students of nursery in Isfahan University of Midwifery. Journal of Training in Medical Science, 2006, 6 (2), 17-25.
5. Farrokhi, F. Quality of performance of midwives in health centers under supervision of Mashhad University of Medical Science. Payesh Journal, 7th year, No. 3, summer 2008: 203-210.
6. Harvey, S.A. Are skilled birth attendants really skilled? A measurement method, some disturbing results and a potential way forward. Bulletin of the World Health Organization. October 2007. 85(10) :733-820.
7. Harvey, S.A. Avabaca, P., Bucaqu, M. Skilled birth attendant competence: An initial assessment in four countries and implications for the safe motherhood movement. Int. J. Gynecol Obstet. November 2004. 87(2): 203-10.
8. Wallace, M. The European Union standards for nursing and midwifery: Information for accession countries. 2001. Www. Who.dk\nursing.
9. Fleming, V., Holmes, A. Basic nursing and midwifery education programs in Europe. A report to the World Health Organization regional office for Europe. March 2005. http:// www.Who.org.
10. Kruske, S., Darwin, C. Papua, New Guinea midwifery education review final report. WHO and Department of Health, Papua New Guinea. 2006.
11. Modarres, M., et al. Effects of training of applying patagram on quality of midwives job. Hayat Journal. No. 21, summer 2004: 79-87.
12. Nasirian, K.H., et al. Self-assessment of graduates of nursery from acquiring clinical skills for surgery. Journal of Training in Medical Science. Spring and Summer 2006, 6 (1) : 93-100.
13. Rochester, S., Kilstoff , K., Scott, G . Learning from success: Improving undergraduate education through understanding the capabilities of successful nurse graduates. Nurse Today. April 2005. 25(3):181-8.
14. Salehi. Changing training courses for nursery and midwifery. Journal of Nursery-Midwifery Research, Isfahan University of Medicine Science. 2004. 3:23.
15. Salehi. Assessment of performance of graduates of nursery from their point of view and from heads of the departments' points of view in hospitals under supervision of Isfahan University of Medical Science. Journal of Training in Medical Science, No. 4, (2003): 42-47.
16. Seyf, A., et al. Psychology Breeding, Tehran, 2005.
17. Udlis, K.A. Preceptorship in undergraduate nursing education: An integrative review. Journal of Nursing Education, Vol. 47, No. 1. January 2008. 20-29.
18. Wennting, T. L. Planning for effective training: Guide to curriculum development. Tarbiat Modaress Publisher, Tehran. 1997.

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Contribution of NGO's functions to Empowerment of Women in Shiraz, Iran

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Abstract: Millions of women living in developing countries constitute 70 percent of the world's 1.3 billion poor (ILO, 2000). Despite many international agreements affirming women's rights, women are still have more limited access than men to property ownership, credit, training and employment. Gender-based inequalities lead women to lower economic, political and social status, particularly in Asian countries. This study seeks to investigate the contribution of NGO's function to the empowerment of women. This study measures two levels of women's empowerment: individual and collective (community) empowerment. Data are collected from eleven women's NGOs with one hundred and ninety five (195) women who supported by these NGOs in Shiraz. This study used the quantitative approach to answer the objectives of study. The results revealed that NGOs have affected on women's empowerment. The finding of this study has important implications for developing of empowerment particularly women in Iran. As result showed, the women empowered individually and collectively as they involved in NGOs activities. This result will help the women to realizing themselves, and finding their situation at the society.
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Key words: NGOs, individual empowerment, collective empowerment, women

1. Introduction

Globally, women are generally lower in status and power relative to men in job prestige, in economic and political terms. Embedded in all these are the power relations between man and woman. Stein (1997) observed that women are disadvantaged in terms of education, employment, income, access to programs and services, health care, and to benefits of modernization projects such as credit, training and jobs. They are disadvantaged economically, politically, legally and socially relative to men, and this tends to be true in most countries.

In Iran, like many other developing countries, women's access to positions of influence and power is restricted. Their occupational choices are narrower and they must struggle to reconcile activities outside the home with their husband and traditional roles. The statistics show that women represent only 2.8% of parliament's members (i.e, 8 out of 290 members). In other areas of public life women's representation is no better, as the following numbers show: members of town councils (2.66%), holding higher management posts (1.2%), in managerial positions (5.2%), and employed in government, private and public sectors (11.8%) (Tahmasebifar, 2005). This is an indication of a minimal participation of women in power and at decision making levels. It is clear that in Iran, the structure of power is entirely male dominated, placing women at the very lowest levels of decision making despite the rise in their education levels and awareness. Although there have been some improvements in women's participation in town councils, nevertheless the position of women in the country's political institutions is extremely poor. Thus, this situation suggests the unfavorable position of women in Iran (Tahmasebifar, 2005).

In the last decade non-governmental organizations (NGOs) have gained increased attention among scholars and practitioners of development. They are identified as effective and efficient with regard to delivering services to disadvantaged communities; they are also praised for promoting community self-reliance and empowerment through supporting community-based groups, and relying on participatory processes (Korten, 1990; Clark, 1991; Friedmann, 1992; Fowler, 1993; Edwards & Hulme, 1994; Salamon, 1994).

Iranian NGO's can be broadly categorized into two groups. The first group encompasses new and modern so-called "developmental" NGOs. The second group includes the traditional "relief" CBOs and charity societies. However, most of the women's NGOs set up under modern and developmental NGOs. Moreover, there are outstanding examples of modern NGOs in Iran that have been successful in addressing social issues, conducting advocacy efforts, raising public awareness and conducting educational activities and tackling tough social issues, with extensive community participation. So, the question to be asked is that, is there any significant difference between NGO's function and women's empowerment?

In other words, this study seeks to investigate the contribution of NGO's function to the empowerment of women. A considerable amount of literature has been published about the role of NGOs in the

empowerment of women (Korten, 1990; Calman, 1992; Rodriguez, Macinko, & Macinko, 1994; Tandon, 1995; Purushothaman, 1998; Haider & Akhtar, 1999; Page & Czuba, 1999; AhsanUllah, 2003; Magar, 2003; Handy & Kassam, 2006). So far, however, there has been little discussion about type of NGO's functions and empowerment of women. In fact, this study seeks to find out which function of NGOs is effective in empowering women. Past researchers have not generally indicated a relation between NGO's function and women's empowerment. Also, there has been no published survey on this topic in Iran.

Social scientists and development practitioners have long been interested in the conditions that empower women. Empowerment gives women freedom of choice, equal access to domestic and community resources, opportunities and powers. It also enhances their decision-making capacity, knowledge, skills and gives them the necessary self-confidence to be full partners in the developmental process (Vijayanthi, 2002). As argued by Moser (1989) , empowerment is the capacity of women to increase their own self-reliance and internal strength. It is also about the woman's right to determine choices in her life and to influence the direction of change, through the ability to gain control over material and non material resources. The question raised here is that what is the level of women's empowerment in Shiraz, Iran? Thus, first this study seeks to identify the level of women's empowerment and second wants to investigate the factors that can be influenced on empowerment of women. The focus of this study is on empowerment of women by the NGO's activities.

2. Literature Review

Streeten (1997) have mentioned that NGOs have been particularly determined to empower the poor, the weak, and the marginalized to encourage people to take decisions themselves, to become agents, rather than treating them as target groups or passive recipients of benefits.
Furthermore, Desai (2005) accounted some roles for NGOs in order to support the women to challenge customs, ideas and belief which effect unequal gender relations. They are, counseling and support services, awareness raising and advocacy, legal aid and Microfinance.

Rodriguez et al., (1994) state some functions for NGOs such as effective at service delivery; able to promote and motivate local participation; and effective in reaching vulnerable groups such as women and minorities.

According to Haider&Aktar (1999), NGOs central goal is empowering the powerless women or helping them to increase their hidden potentialities, in order to helping them to participate in the mainstream of socio-economic development (Islam & Sultana, 2005).

NGOs do some activities and programs such as funding projects, engaging in service provision and capacity building, contributing to awareness raising, and promoting the self-organization of various groups to contribute the development of a community (Baccaro, 2006). Further, NGOs can promote the empowerment of the poor, particularly poor women, through a combination of micro-credit, awareness-raising, and training for group members (Ramesh, 1996).

Empowerment is a process whereby women become able to organize themselves to increase self-reliance and to assert their independent right to make choices and control resources. It is a participatory process of awareness and capacity building that begins at the levels of household and community, reading to greater participation and decision-making power and control, and to transformative action enabling individuals or groups to change balances of power in social, economic and political relations in society (Karl, 1995).

This study constructs two components of empowerment, namely individual empowerment and community empowerment, relying mostly on Rowlands (1997) and Schulz, et al., (1993) as their work seems most relevant to the objectives of this study.

Personal empowerment views empowerment as focused on individual strength and self-esteem to gain control over available resources and to exercise their right to obtain quality of life for themselves and their family (Moyle, Dollard, & Biswas, 2006). Individual empowerment includes the following elements: Self-esteem, decision-making ability, and control over life situations (Rowlands, 1998). There is much support for choosing these particular elements.

A community empowerment is one in which individuals apply their skills and resources in collective efforts to meet their respective needs. Through such participation, individuals provide enhanced support for each other, address conflicts within the community, and gain increased influence and control over the quality of life in their community (Schulz et al., 1993). As Rowlands (1997) pointed out, collective empowerment involves individuals working together to achieve a greater impact than they could have alone. Community empowerment includes some elements such as working together, participation in NGO's program and community awareness.

3. Methodology

This study used quantitative approach in the inquiry process to understand and explain empowerment which could be investigated and measured objectively. Survey research design, is a procedure in quantitative approach which help the researchers to administer a questionnaire in order to identify trends in the attitudes, opinion, behavior or characteristic of population (Creswell, 2005). In this study since the empowerment as a major issue of the study, is measurable and accountable with its elements, thus the survey technique using questionnaire was implemented.

For purpose of this study, the target populations were women who involved in NGOs in Shiraz. They were around 2000 of 11 NGOs in city of Shiraz in terms of different type and activity. To determine the proportion of each NGO in sample size, it used of sampling ratio and in order to selection of respondents it used of simple random sampling technique.

A structured self administered questionnaire was developed as the mod of data collection. This survey method was adopted because the administration of questionnaire incurs less cost and saves time compared to the face-to-face interview and telephone interview (Ary et al., 2006). Beside, questionnaires are used to gather a large number of quantitative responses. The respondents were told in advance that they could stop any time if they felt uncomfortable about completing questionnaire. Also, the interviewer read the questionnaire items for women who were illiterate. Women were asked if they had any questions about the questionnaire and told that their responses would remain confidential. First of all, the respondents were asked questions which evaluated elements of individual empowerment such as; self-esteem, decision-making ability, and control over life. The assessment was followed by the elements of community empowerment which included working together, community awareness, and participation in NGO's programs.

The finalized questionnaire was administered to a group of 30 respondents who were randomly selected to take part in this pilot study. Then, the data were analyzed and the Cronbach's alpha coefficient of reliability was derived. According to Garson (2009), the dimensions should have a Cronbach's alpha of at least .70 to establish reliability of constructs. The reliability index Cronbach's alpha of individual empowerment with 32 items was .832, community empowerment with 25 items was.804.Finally, the data analysis showed that reliability index Cronbach's alpha for women's empowerment(Total empowerment) with 57 items scale and five point scale was.780.

All data from this study was coded and entered in a computer files by the author. Identification information of respondents (their name) was not included on questionnaire. Data collection took place over a period of two month in the first semester of 2008-2009. It began in November, 2009 and ended in December, 2009.

Data obtained from the respondents were coded, computed and analyzed using statistical package for the social science SPSS (version 16). They were analyzed using a combination of statistical analysis such as, descriptive statistics, and ANOVA test. A variety of descriptive statistics were utilized for example, frequency distribution and measures of central tendency. ANOVA test was used to determine the significance in women empowerment between each group of NGO's functions.

4. Results

4.1 Individual empowerment

In this study women's empowerment is operationalized at level of individual and community empowerment in which constituted six elements to measure their empowerment level. For individual level, three elements were self-esteem, decision-making ability, and control over life whereas, for the community level, the elements were working together, participation in NGOs' program, and community awareness.

Individual empowerment in this study was measured by 32 items, five point likert scales. To calculate the individual empowerment, three dimensions were computed. They were self-esteem, decision-making ability and control over life. Individual empowerment is ranged from 84 to 143 with the mean score of M=120.68 and standard deviation SD= 9.13. The skewnes (-.26) showed the distribution of variable was normal. Further, data reported in Table 1 indicated that the majority of respondents (60.5%) were at the moderate level of individual empowerment. This followed by 25.1% at the high level and only 12.3% reported low level of individual empowerment. The result indicated that individual empowerment was at the moderate level among the Iranian women.

Table 1: level of individual empowerment (N=195)

levels	frequency	%
Low(84-103)	24	12.3
Moderate (104-123)	118	60.5
High (124-143)	49	25.1

4.2 Community (collective) empowerment

Community (collective) empowerment was comprised of community awareness, working together, and participation in NGOs' program.

Community empowerment score ranged from 73 to 119 with the mean score of M=102.25 and standard deviation SD=7.97. Distribution of data based on skewnes (-.23) reported was normal. Table 2 represented the frequency of score on community empowerment by the overall sample. The most of respondents (52.3%) were at moderate level of collective empowerment and 43.6% reported at the high level of empowerment. However, only 3.6% mentioned as the low level of community empowerment. These findings showed that the score of collective empowerment among the women was at the moderate level and tend to be high. Furthermore, result indicated that the mean score of individual empowerment (M=120.68) among the women in Shiraz was higher than (collective) community empowerment (M=102.25). This probably means that after joining NGO, many of the respondents experienced personal empowerment, improved their sense of control over their own lives, raised their self-esteem, and increased their ability of decision-making.

Table2: Level of community empowerment

Levels	frequency	%
Low(73-88)	7	3.6
Moderate (89-103)	102	52.3
High (104-119)	85	43.6

4.3 Women's empowerment

Women's empowerment in this study comprised of two level, individual and community level. To measure the empowerment, first we calculate individual level in which had three elements. Then, we accounted the community or collective level which included three elements. Finally, these two levels have been computed as a women's empowerment. It is ranged from 168 to 261 with the mean score of M= 222.94, and standard deviation was SD=14.31.The skewness (.01) showed that the distribution of dependent variable was normal.

The percentage of women empowerment depicted in Table 3 showed that the majority of respondents (59.5%) were at moderate level of empowerment. About 21.5% reported on high level of empowerment and 19% indicated at the low level of empowerment. These finding indicated that the majority of women in this study had the moderate level of empowerment.

Table 3: level of women's empowerment

Variable	Frequency	%
Low (186-210)	37	19
Moderate (211-235)	116	59.5
High (236-261)	42	21.5

The finding revealed that the level of women's empowerment among the Iranian women was at the moderate level. The result showed that the mean score of total empowerment was, M=222.94 and standard deviation was, SD=14.31. However, taking into account the socio-cultural background of the country which has been a large traditional practicing conservative way of live male dominated social structure, a little change as experienced by the women shown in this study is a positive indication. In other words, women despite of social, cultural and political constraints they could gain a little power to improve their situation of lives.

4.4 The effect of NGOs' functions on individual empowerment

One-way ANOVA was used to assess the mean difference of individual empowerment among NGO's functions. NGO's function as depicted in Table 4 were 'developing individual ability' 'educational functions' ' awareness building' and ' mobilizing of women'. The results of ANOVA test revealed that there was a statistically significant difference in the mean of individual empowerment for NGO's functions among four functions, F (3, 191) =3.86, P=.01. It means that there is a difference among NGO's functions on individual empowerment.

Table 4: One-Way ANOVA Summary Table of NGO's functions and its Scores

Functions	N.NGOs	N	Mean	SD	F	P	η^2
Developing individual ability	2	45	123.57	8.69	3.86	.01	.057
Educational functions	3	55	118.80	8.15			
Awareness building	3	35	118.14	9.39			
Mobilizing women	3	60	121.11	9.52			

Furthermore, the post hoc multiple comparisons were selected to evaluate pairwise differences among mean score of individual empowerment in NGO's functions. The result of Tukey test showed that the significant mean score difference existed between following pairs; developing individual ability and educational functions, and developing individual ability and awareness building. Tukey test indicated mean scores of developing individual ability (M=123.57, SD=8.69) was significantly higher than educational functions (M=118.80, SD=8.15). Developing individual ability also was significantly higher than awareness building (M=118.14, SD=9.39).Thus, the results show that the function of 'developing individual ability' has higher mean score of individual empowerment compare to the other functions.Table 5 showed these results.

Developing individual ability as a function of NGOs refers to programs such as building self-esteem of women through some activities as

mentioned by NGO's leadership and increase the ability of women to make decision regarding their children, household and their life. All these activities, functions and programs are toward the individual ability and capability of women. On the other hands, individual empowerment was measured through some elements such as self-esteem, decision-making ability and control over life. Thus, it can be concluded, the function of developing individual ability has higher effect on individual empowerment. It means that in Shiraz those NGOs have this function had higher contributed to individual empowerment.

Table 5: Result of post hoc comparison of individual empowerment and NGO's functions

Dependent variable	NGO's functions	NGO's functions	Mean difference	Sig
Individual empowerment	Mobilizing women	Educational functions	2.31	.57
		Awareness building	2.96	.45
		Developing individual ability	-2.45	.50
	Educational functions	Mobilizing women	-2.31	.57
		Awareness building	.65	.98
		Developing individual ability	-4.76*	.02
	Awareness building	Mobilizing women	-2.96	.45
		Educational functions	-.65	.98
		Developing individual ability	-5.42*	.02
	Developing individual ability	Mobilizing women	2.45	.50
		Educational functions	4.76*	.024
		Awareness building	5.42*	.025

4.5 The effect of NGOs' functions on community empowerment

ANOVA test was performed to investigate the difference in the mean of community empowerment score on NGO's functions. As Table 6 presented from the statistical test, it was revealed that there was significant differences in the mean of community empowerment among four functions of NGOs, F (3, 191) =2.58, P=.04. The eta–squared of.05 confirmed that mean differences among the four functions were fairly moderate. Analysis of mean showed that mobilizing women has the higher mean (M=103.87, SD=7.86) rather than the others functions. It means the function of mobilizing women has more effective toward community empowerment compare to other NGO's functions.

Table 6: One-Way ANOVA Summary Table of NGO's functions and its Scores

Functions	N.NGOs	N=195	Mean	F	P	η^2
Developing individual ability	2	45	102.3	2.58	.04	.05
Educational functions	3	55	101.56			
Awareness building	3	35	100.43			
Mobilizing women	3	60	103.87			

This is may be because of the nature of mobilizing women as a function of NGOs. Through mobilizing function, NGOs encourage women to participate in programs in order to identify the community's problem and to find out a solution for the community. As Panda (2007) noted by encouraging people to participate in their projects, NGOs build a rapport with the local people and seek to understand their problems. They tend to involve people in the different stages of the projects, starting from the project initiation to project implementation and maintenance. On the other hand, the elements of community empowerment implied the cooperation and participation of respondents in the programs. Furthermore, according to leadership of NGOs which have this function, they encourage women to participation through some activities and programs such as giving the reward to women, preparing the opportunities for women, and explaining the benefit of programs. Therefore, it can be said that this function has the higher contribution to community empowerment.

4.6 The effect of NGOs' functions on women's empowerment

Women's empowerment in this study as mentioned earlier, included two levels that is individual and collective empowerment. After that these two levels were calculated and women's empowerment was derived.

One-way ANOVA was employed to distinguish the difference of the mean of the women's empowerment score among the four functions of NGO namely developing individual ability, educational functions, awareness building, and mobilizing women. The results of ANOVA test as depicted in Table 7 there was a statistically significant difference in the mean of women's empowerment among four functions of NGOs, F(3, 191) =3.83, P=.01.

Furthermore, since the number of cases not equal in each item the post hoc multiple comparison were selected to evaluate pairwise differences of the mean score of empowerment among the NGO's functions. The result of Tukey test showed that the significant mean score difference existed between mobilizing women and awareness building, and mobilizing women and educational functions since $p<.05$ were reported for the four functions.(Table 8). These comparative result suggested that the mobilizing women as NGO's function had high level of performance (M=227.43, SD=15.59), followed by developing individual ability (M=223.49, SD=12.65) and educational functions (M=222.36, SD=14.48). Based on Cohen (1988) criteria (.01=small effect,

.06=moderate effect, and .14=large effect), the effect size (eta-squared) of NGO's functions as independent variable toward women's empowerment as dependent variable was .057 indicating that mean differences among functions were moderate.

Table 7: One-Way ANOVA Summary Table of NGO's functions and its Scores

Functions	N.NGOs	N=195	Mean	F	P	η^2
Developing individual ability	2	45	223.49	3.83	.01	.057
Educational functions	3	55	220.36			
Awareness building	3	35	218.57			
Mobilizing women	3	60	227.43			

Table8: Result of Post hoc multiple comparisons of NGO's functions and women's empowerment

Dependent variable	NGO's functions	NGO's functions	Mean difference	Sig
Women's empowerment	Developing individual ability	Educational functions	3.12	.68
		Awareness building	4.91	.40
		Mobilizing people	-3.94	.48
	Educational functions	Developing individual ability	-3.12	.68
		Awareness building	1.79	.93
		Mobilizing people	-7.06*	.03
	Awareness building	Developing individual ability	-4.91	.40
		Educational functions	-1.79	.93
		Mobilizing people	-8.86*	.01
	Mobilizing people	Developing individual ability	3.94	.48
		Educational functions	7.06*	.03
		Awareness building	8.86*	.01

*. The mean difference is significant at the 0.05 level.

According to Ramesh (1996), Stromquist(2002) and the others, similarity in Shiraz the NGO do performed some functions such as developing the individual ability, educational functions, awareness building and mobilizing of women to participate in projects.

Mobilizing people or people participate in project is an important strategy adopted by NGOs. NGOs, not only encourage people to participate in the activities and programs carried out by them, but also to mobilize resources both in cash and kind. They tend to involve people in the different stages of the projects, starting from the project initiation to project implementation and maintenance (Panda, 2007). As Streeten (1997) mentioned, NGOs have been particularly determined to empower the poor, the weak, and the marginalized, to encourage people to take decisions themselves, to become agents, rather than treating them as target groups or passive recipients of benefits.

5. Discussions

The present study was intended to investigate the empowering of women through NGO's efforts. The result of this study showed that the level of women's empowerment was at the moderate among the women in Shiraz Iran. It means that they were never disempowered nor empowered sufficiently at the individual and community level. Considering as well, the socio-cultural background of the country which has been a large traditional practicing conservative way of live and a male dominated social structure, a little change as experienced by the women shown in this study is a positive indication. On the other hand, NGOs particularly women's NGO is a new phenomenon in Iran which are active in a variety of field to address some issues such as social needs, raising public awareness and conducting educational activities and tackling tough social issues with extensive community participation. Therefore, it can be concluded that NGOs in Shiraz could be contributed in empowerment of women. Although, the NGOs those focus on function of 'mobilizing women' had the more effectiveness in empowerment. Previous research focused little attention to this relationship of NGOs' functions and empowerment. Generally, in Iran women are not welcome to get involve in social, economic and political activities. Thus, those NGOs which could encourage women to participate in programs will be successful in the empowering process.The present study showed that the NGOs played important role in empowering the women as shown in the finding. These findings are consistent with prior researches that claimed NGOs have a role in facilitating empowerment processes (Korten, 1990; Calman, 1992; Tandon, 1995; Puroshothaman, 1998; Page & Czuba, 1999).

To conclude, since the study is based on the community development and the empowerment of women and improvement of their status are viewed as essential for the achievement of such development. Further, NGOs function and activities were considered as the important factors to empowerment. The study suggest by improving women own lives, women can contribute to the development of the community as well as in nation-building. Therefore, the study contributes to the development of the community as well as nation building.

The finding of this study has important implications for developing of empowerment of women in Iran. The result showed that the women were empowered individually and collectively as they involved in NGOs activities. This result will help the women to realizing themselves, and finding

their situation at the society. It is suggested that women need to engage in various activities that organized by NGOs. Through their involvement in activities of NGOs, women could access the knowledge, learned the skills, got the job, and accessed the power and control in order to improve of their lives.

On the other hand, local NGOs as a third sector in Iran despite of some problems such as financial, political and traditional could be benefited to improve the women situation in Iran. The study suggests that the government and governmental agencies should to take the necessary steps to provide financial and social support for NGOs so that they could organize their activities.

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REFERENCES

1. AhsanUllah, A. (2003). Empowerment of Women in Bangladesh: Do NGO Interventions Matter? *Empowerment: Women for Women, Dhaka, 10*.
2. Ary, D., Jacobs, L., Razavieh, A., & Sorensen, C. (2006). *Introduction to Research in Education*. CA: Wadsworth Pub Co.
3. Baccaro, L. (2006). Civil Society Meets the State: Towards Associational Democracy? *Socioecon Review, 4*(2), 185-208.
4. Calman, L. (1992). *Toward Empowerment: Women and Movement Politics in India*. Boulder, Co: Westview Press.
5. Clark, J. (1991). *Democratizing Development: The Role of Voluntary Organizations*. West Hartford: Kumarian Press.
6. Cohen, J. (1988). *Statistical Power Analysis for the Behavior sciences* (2 ed.). New Jersey: Hills Dale.
7. Creswell, J. W. (2005). *Educational Research: Planning, Conducting and Evaluating Quantitative and Qualitative Research*. New Jersey: Pearson.
8. Cronbach, L. (1951). Coefficient Alpha and the Internal Structure of Tests. *Psychometrika, 16*(3), 297-334.
9. Desai, V. (2005). NGOs, Gender Mainstreaming, and Urban Poor Communities in Mumbai. *Gender and Development, 13*(2).
10. Edwards, M., & Hulme, D. (1994). *NGOs and Development: Performance and Accountability in the 'New World Order'*. Paper presented at the International Workshop on NGOs and Development, University of Manchester.
11. Fowler, A. (1993). Non-Governmental Organizations as Agents of Democratization: An African perspective. *Journal of international Development, 5*(3).
12. Friedmann, J. (1992). *Empowerment: The Politics of Alternative Development*. Cambridge: Blackwell.
13. Garson, D. (2009). Structural Equation Modeling. Retrieved September, 2009, from http://faculty.chass.ncsu.edu/garson/PA765/structure.htm.output
14. Haider, R., & Akhtar, R. (1999). The Role of NGO and Women's Perception of Empowerment: An Anthropological Study in a Village. *Empowerment: Women for Women, Dhaka, 6*.
15. Handy, F., & Kassam, M. (2006). Practice What you Preach? The Role of Rural NGOs in Women's Empowerment. *Journal of Community Practice, 14*(3), 69-91.
16. Islam, N., & Sultana, N. (2005). Role of NGOs in Empowering the Vulnurable Women: A Study on ACD. *Asian Affairs, 27*(4), 24-40.
17. Karl, M. (1995). *Women and Empowerment: Participation and Decision Making*. London: Zed Books.
18. Korten, D. C. (1990). *Getting to the 21st Century : Voluntary Action and the Global Agenda*: West Hartford, Conn: Kumarian Press.
19. Magar, V. (2003). Empowerment Approaches to Gender-Based Violence: Women's Courts in Delhi Slums. *Women's Studies International Forum, 26*(6), 509-523.
20. Moser, C. (1989). Gender Planning in the Third World: Meeting Practical and Strategic Gender Needs. *World Development, 17*(11), 1799-1825.
21. Moyle, T., Dollard, M., & Biswas, S. (2006). Personal and Economic Empowerment in Rural Indian Women: A Self-help Group Approach. *International Journal of Rural Management, 2*(2), 245.
22. Page, N., & Czuba, C. E. (1999). Empowerment: what is it? *Journal of Extension, 37*(5), 1-5.
23. Panda, B. (2007). Top Down or Bottom Up? A Study of Grassroots NGOs' Approach. *Journal of Health Management, 9*(257).
24. Purushothaman, S. (1998). *The Empowerment of Women in India: Grassroots Women's Networks and the State*. New Delhi: Sage Publications
25. Ramesh, J. (1996). Strategies for Monitoring and Accountability. The Working Women's Forum Model. In M. Edwards & D. Hulme (Eds.), *Beyond the Magic Bullet - NGO Performance and Accountability in the Post-Cold World.Save the Children*. West Hartford: CT, Kumarian Press.
26. Rodriguez, G. R., Macinko, J., & Macinko, M. (1994). The Role of NGOs in Community Health and Development. *Promotion and Education, 1*(4), 5.
27. Rowlands, J. (1997). *Questioning Empowerment: Working with Women in Honduras*. New Jersey: Oxfam
28. Rowlands, J. (1998). A Word of the Times, but What Does it Mean? Empowerment in the Discourse and Practice of Development in Afshar Haleh (Ed) Women and Empowerment: Illustrations from the Third World.
29. Salamon, L. (1994). The Rise of the Nonprofit Sector. *Foreign Affairs, 73*(4), 109-122.
30. Schulz, A. J., Israel, B. A., Zirmmerman, M. A., & Checkoway, B. N. (1993). *Empowerment as a Multi-Level Construct: , Perceived Control at the Individual, Organizational and Community Levels*. Paper presented at the Program on Conflict Management Alternatives.
31. Solomon, B. (1976). *Black Empowerment: Social Work in Oppressed Communities*. New York: Columbia University Press.
32. Stein, J. (1997). *Empowerment& Women's Health*. London: Zed Books.
33. Streeten, P. (1997). Nongovernmental Organizations and Development. *Annals of the American Academy of Political and Social Science, 554*, 193-210.
34. Stromquist, N. P. (2002). NGOs in a New Paradigm of Civil Society. *Current Issues in Comparative Education, 1*(1).
35. Tahmasebifar, S. (2005). *Situation Analysis of Women in Iran 10 years after Beijing*: Asia Pacific Women.
36. Tandon, R. (1995). Board games: Governance and accountability. In M. Edwards & D. Hulme (Eds.), *NGO performance and accountability*. London: Earthscan.
37. Vijayanthi, K. (2002). Women's Empowerment Through Self-help Groups: A Participatory Approach. *Indian Journal of Gender Studies, 9*(2), 263.

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Colibacillosis in Newly Born Buffalo Calves and Role of Lacteol Fort in Preventing Recurrence of Calf Diarrhea

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Abstract: *Escherichia coli* diarrhea (scours) of newborn calves is characterized by watery white or yellowish diarrhea, rapid onset, and high mortality. The objectives of this study were twofold. First, the role of *E. coli* infection in diarrheic newborn buffalo calves was assessed through analysis of clinical data, morbidity and mortality rates, presence of varying *E. coli* serotypes, and antimicrobial sensitivity of bacterial populations in diarrheic fecal samples. Second, we assessed the role of probiotic supplementation with Lacteol Fort in prevention of reoccurrence of calf diarrhea. Our study population consisted of 130 diarrheic newborn buffalo calves, aged 1–11 days, 35–45 kg in body weight, suckling normally during the winter season. The incidence of *E. coli* infection in diarrheic newborn calves was 39.23% (51 of 130 calves). The mortality rate in these infected calves was 25.49% (13 of 51 calves). Clinical findings in calves infected with *E. coli* varied, and included fever, growth depression, recumbency, dehydration, profuse watery yellowish diarrhea in some cases, profuse watery to pasty foul-smelling diarrhea in other cases, and occasional occurrence of yellowish to white diarrhea streaked with blood. The most common *E. coli* serotypes (with rate of detection) were O26 (23.52%), O103 (19.6%), and O119 (17.64%). Antimicrobial sensitivity tests on 51 fecal samples revealed that *E. coli* present in fecal samples was most sensitive (with rate of sensitivity) to marbofloxacin (96.07%), enrofloxacin (88.23%), cefotaxime (84.31%), amoxicillin (78.43%) and spectinomycin (33.33%) and more resistant to penicillin, neomycin, erythromycin, streptomycin, and chloramphenicol. The cure rate in calves supplemented with Lacteol Fort was 80% compared to 69.23% in calves in the unsupplemented group. The recurrence rate of diarrhea, assessed 15 days post treatment, was 8% in the Lacteol Fort group, compared with 26.92% in the unsupplemented group. We conclude that probiotic supplementation with Lacteol Fort can significantly reduce the recurrence of diarrhea in newborn buffalo calves.
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1. Introduction

Diarrhea is a major problem in livestock production in Egypt and throughout the world (**Farid *et al.*, 2001 and Ibrahim, 2007**). Enteritis in newborn calves causes high morbidity and mortality, leading to significant economic losses in Egypt (**Novert and Hammad, 2001 and Ashraf, 2007**). Diarrhea due to *Escherichia coli* (also known as scours) is one of the most common diseases of young calves, despite vaccination programs and management measures, necessitating treatment with antibiotics and fluid therapy (**Gyles, 1993**). *E. coli* diarrhea in newborn calves (9–10 days of age) is usually characterized by watery white or yellowish diarrhea, rapid onset and time course, and high mortality. In affected calves, diarrhea typically begins within 36–72 hours of birth, and affected calves die within 2–3 days. Some calves die several hours after appearing healthy and free of diarrhea.

Calf scours is not a single disease entity; it is a clinical syndrome associated with several diseases characterized by diarrhea. Regardless of the cause, absorption of fluids from the intestine is altered, leading to life-threatening electrolyte imbalances. The scouring calf loses fluids, rapidly dehydrates, and suffers from electrolyte loss and acidosis. Infectious agents may cause initial damage to the intestine, but death from scours usually results from dehydration, acidosis, and loss of electrolytes. Identification of infectious agents that cause scours is essential for implementation of effective preventive and treatment measures (**Radostits *et al.*, 2007**).

E. coli populations are divided into serotypes and serogroups on the basis of antigenic composition (somatic or O antigens, flagellar or H antigens, and capsular or K antigens; Campos ***et al.*, 2004**). In one study, the most common *E. coli* serotypes isolated from diarrheic fecal samples were O119, O111, O126, and O78 (**Tamaki *et al.* , 2005**). **Badouei *et al.* (2010)** isolated O157:H7, O111 and O26 serotypes of *E. coli* strains from 297 fecal samples, from 200 diarrheic and 97 non-diarrheic calves. The most predominant serogroup was O26 (18.4%).

Current treatment regimens for the treatment of neonatal calf diarrhea center on antimicrobial therapy and fluid therapy. Recently, there has been increased

use of probiotics in treatment of neonatal calf diarrhea. Probiotics support the balance of intestinal flora, inhibit epithelial adhesion and invasion by pathogenic bacteria, and produce antimicrobial substances that are inhibitory to both gram positive and gram-negative bacteria (**Rolfe, 2000**). Probiotics are bacterial preparations that benefit the host by improving microbial balance and eliminating or reducing pathogenic microorganisms. In the case of diarrhea, a potential effect of probiotic bacteria is the production of enzymes that inactivate enterohemorrhagic *E. coli*, reducing its colonization of the gut and consequently lowering shedding of the pathogen (**Hughes *et al.*, 2010**).

The lactobacilli in Lacteol Fort are able to adhere to intestinal cells. By inhibiting the ability of pathogens to adhere to and invade intestinal cells, Lacteol Fort blocks the infectious process of diarrhea and stops symptoms rapidly (***Chauviere, 1992 and Coconnier, 1997***).

The aims of our study were twofold. First, we sought to determine the incidence of E. *coli* infection in diarrheic newborn buffalo calves and to examine clinical findings, morbidity and mortality rates, *E. coli* serotypes, and antimicrobial sensitivity of bacteria in diarrheic fecal samples. Second, we aimed to examine the role of Lacteol Fort in preventing the recurrence of calf diarrhea.

2. Material and Methods

A. Animals:

Our study population consisted of 130 buffalo calves from a dairy breeding buffalo farm at El-Bahiera Governorate, Egypt, aged 1–11 days, 35–45 kg in body weight, suckling normally during the winter season. Dams were not vaccinated against enteric infection. Of the 130 calves, 51 suffered from diarrhea.

B. Clinical examination and collection of samples:

Calves were examined clinically by veterinarian, and morbidity and mortality rates were documented. For assessment of colibacillosis, rectal swabs were taken from diarrheic calves using sterile cotton swabs (Boyd *et al.,* 1974) and kept on ice before storage in the laboratory.

C. Isolation and identification of *E. coli*:

Bacterial methods were previously described (Cruickshank *et al.,* 1975; Koneman *et al.,* 1992; and Quinn *et al.,* 1994).

D. Serological identification of *E. coli* isolates:

E. coli isolates from fecal samples were analyzed using monospecific antisera (SSI Diagnostica, Hillerød, Denmark) as previously described (Ewing, 1986). See Table 1 for a list of sera used in the analysis. Serological tests were performed by the Department of Clinical Microbiology, Central Health Laboratories, Ministry of Health, Cairo, Egypt.

Table 1. Monospecfic sera used in serological identification of *E. coli*

Anti-coli O25:(K11)	Anti-coli O114:(K90)
Anti-coli O26:(K60)	Anti-coli O118:(K-)
Anti-coli O44:(K74)	Anti-coli O119:(K69)
Anti-coli O55:(K59)	Anti-coli O124:(K72)
Anti-coli O78:(K80)	Anti-coli O125:(K70)
Anti-coli O86:(K61)	Anti-coli O126:(K71)
Anti-coli O91:(K-)	Anti-coli O127:(K63)
Anti-coli O25:(K11)	Anti-coli O128:(K67)
Anti-coli O103:(K-)	Anti-coli O142:(K86)
Anti-coli O111:(K58)	Anti-coli O157:(K-)

E. Antimicrobial sensitivity test:

Assessment of antimicrobial sensitivity in fecal isolates was performed as previously described (Quinn *et al.,* 1994). Results were interpreted according to Koneman *et al.,* 1992 (Table 2 for parameters).

Table 2. Parameters used in assessing antimicrobial sensitivity

Antimicrobial agent	Disc content	Inhibition zone diameter (mm)		
		Susceptible	Intermediate	Resistant
Marbofloxacin	10 µg	>30	20–24	<20
Enrofloxacin	10 µg	>25	14–15	<12
Gentamycin	10 µg	>15	13–14	<12
Erythromycin	15 µg	>18	14–17	<13
Cefotaxime	30 µg	>18	15–17	<14
Amoxicillin	10 µg	>29	21–28	<19
Penicillin	10 units	>29	20–28	<20
Tetracycline	30 µg	>19	15–18	<14
Chloramphenicol	30 µg	>18	13–17	<12
Streptomycin	10 µg	>15	12–14	<11
Trimeth/sulfa	1.25/23.75 µg	>16	11–15	<10
Spectinomycin	20 µg	>15	12–13	<10
Neomycin	20 µg	>18	15–17	<14

Antimicrobial sensitivity was assayed as described in (Koneman et al., 1992) and categorized as indicated in the table. Trimeth/sulfa: trimethoprim/sulfamethoxazole.

F. Treatment of *E. coli* diarrhea:

Calves were treated for diarrhea with the following reagents:

1. Antimicrobial therapy: Enroflox 10%, containing 10% enrofloxacin (El-Nasr, Egypt); and Finadyne, containing 50 mg/ml flunixin meglumine (Schering- Plough Animal Health, Germany).
2. Oral rehydration salts: Each sachet of 5.5 g contains glucose anhydrous 4.00 g, trisodium citrate anhydrous 0.51 g, sodium chloride 0.70 g, and potassium chloride 0.30 g (Elamria Co., Egypt).
3. Probiotics: Lacteol Fort; each sachet contains 10 billion lactobacilli (*Lactobacillus delbruekii* and *Lactobacillus fermentum*; Rameda, Egypt).
4. Electrolyte therapy: Equal mixture of isotonic saline (85%), isotonic sodium bicarbonate (1.3%) and isotonic dextrose (5%).

The 51 diarrheic calves were classified into 2 treatments groups as follows.

1. Unsupplemented group (26 calves)

Enroflox 10%, 1 ml/20–40 kg body weight, i.m. for 3 successive days.
Oral rehydration therapy.
Finadyne, 2 ml/45 kg body weight, i.v. or i.m.
Fluid therapy: 1–3 liter fluid/calf, delivered 50–100 ml/kg body weight, i.v.
Milk intake reduced for up to 24 hours until clinical improvement.

2. Lacteol Fort group (25 calves)

Treatment was identical to that in the unsupplemented group, with the addition of probiotics: Lacteol Fort 3 sachets daily for 3 successive days.

3. Results

Table 3. Incidence of *E. coli* in diarrheic buffalo calves

Age	No. of calves examined	No. of calves infected with *E. coli*	% of calves with *E. coli* infection
3–4 days	46	14	30.43
5–7 days	32	21	65.62
8–11 days	52	16	30.76
Total	130	51	39.23

$\chi^2 = 5.85$ * *Significant at P = 0.05

Table 4. Morbidity and mortality in calves infected with *E. coli*.

Age	No. of infected calves	Morbidity rate (%)	No. of dead calves	Mortality rate (%)
3–4 days	14	30.43	6	42.85
5–7 days	21	65.62	4	19.04
8–11 days	16	30.76	3	18.75
Total	51	39.23	13	25.49

$\chi^2 = 20.87$ *** *** Significant at P < 0.001

Morbidity and mortality are expressed as percentages of the total number of calves (diarrheic and unaffected) in each group.

Table 5. Clinical findings in diarrheic calves

Age (number of calves)	Clinical observations
3–4 days (14)	Six calves suffered from fever, growth depression, recumbency, dehydration, profuse watery yellowish diarrhea with foul odor, followed by death. Eight calves suffered from profuse watery to pasty diarrhea with foul odor, and refuse to suckle.
5–7 days (21)	Four calves suffered from profuse watery diarrhea, usually yellow to white color (sometimes streaked with blood) with foul odor, dehydration, recumbency, followed by death. Seventeen calves suffered from pasty diarrhea with foul odor.
8–11 days (16)	Three calves suffered from yellowish watery diarrhea, dehydration, recumbency with subnormal temperature, followed by death. Thirteen calves suffered from pasty diarrhea with foul odor.

Table 6. Prevalence of *E. coli* serotypes in fecal samples from diarrheic calves.

Serotype	Number of samples containing serotype (total 51)	Prevalence of serotype in diarrheic samples
O26	12	23.52
O44	2	3.92
O78	1	1.96
O86	3	5.88
O103	10	19.6
O111	3	5.88
O119	9	17.64
O125	2	3.92
O128	2	3.92
O157	3	3.92
Untyped	4	7.84

$\chi^2 = 22.55$ ** ** Significant at P < 0.01

Prevalence is expressed as a percentage of the total number of diarrheic calves.

Table 7. Antimicrobial sensitivity of bacteria in fecal samples from diarrheic calves.

Antimicrobial agent	Susceptible (S)		Moderately susceptible (M)		Resistant (R)	
	No. of samples	% of total	No. of samples	% of total	No. of samples	% of total
Marbofloxacin	49	96.07	2	3.92	0	0
Enrofloxacin	45	88.23	4	7.84	2	3.92
Gentamycin	9	17.64	25	49.01	17	33.33
Erythromycin	1	1.96	6	11.76	44	86.2
Cefotaxime	43	84.31	4	7.84	4	7.84
Amoxicillin	40	78.43	7	13.72	4	7.84
Penicillin	-	-	-	-	51	100
Tetracycline	4	7.84	19	37.25	28	54.90
Chloramphenicol	3	5.88	22	43.13	26	50.98
Streptomycin	3	5.88	13	25.49	35	68.62
Trimeth/sulfa	7	13.72	24	47.05	20	39.21
Spectinomycin	17	33.33	18	35.29	16	31.37
Neomycin	-	-	13	25.49	38	74.50

Sensitivity is expressed as a percentage of the total number of diarrheic calves.

Table 8. Cure rate and recurrence rate in diarrheic calves after different treatments.

Group	Cure rate from diarrhea		Recurrence of diarrhea	
	No.	%	No.	%
Unsupplemented group (26 calves)	18	69.23	7	26.92
Lacteol Fort group (25 calves)	20	80	2	8

$\chi^2 = 1.55^{***}$ *** Significant at $P < 0.001$

Rates are expressed as percentages of the total number of calves in each group.

4. Discussion

Incidence of *E. coli* in diarrheic newborn buffalo calves was 39.23% (51 of 130 calves). As shown in Table 3, incidence varied by age group, with calves aged 5–7 days showing much higher incidence (65.62%) than calves aged 3–4 days (30.43%) and 8–11 days (30.76%). Incidence of *E. coli* diarrhea in newly born calves was significantly different according to age at $P = 0.05$. These results suggest that calves aged 5–7 days were more susceptible to *E. coli* diarrhea than other age groups. Notably, *E. coli* was the most common bacteria isolated from fecal samples of diarrheic calves in previous studies (China *et al.,* 1998 and Harbby, 2002).

Morbidity rate and mortality rate in diarrheic calves infected with *E. coli* also showed age dependence. Overall morbidity was 39.23% (51 of 130 calves), and overall mortality was 25.49% (13 of 51 calves). As shown in Table 4, mortality rate was the highest (42.85%) in calves aged 3–4 days; mortality was much lower in calves aged 5–7 days (19.04%) and 8–11 days (18.75%). These results were significantly different according to age at $P < 0.001$. Enteritis in newborn calves is known cause high morbidity and mortality rates, leading to significant economical losses in Egypt (Novert and Hammad, 2001 and Ashraf, 2007).

Clinical findings in calves infected with *E. coli* are presented in Table 5. Our findings are consistent with previous reports on *E. coli* diarrhea (scours) in newborn calves (9–10 days of age). Scours is usually characterized by watery white or yellowish diarrhea, rapid onset and time course, and high mortality. In affected calves, diarrhea typically begins within 36 to 72 hours after birth, and the calves die within 2 to 3 days (El- Sawalhy, 1999, Radostits, *et al.,* 2007 and Mahmoud, 2009).

As shown in Table 6, the most common *E. coli* serotypes in isolated samples (and their prevalence) were O26 (23.52%), O103 (19.6%), and O119 (17.64%). Other serotypes detected (and their prevalence) were as follows: O86, O111, and O157 (5.88%); O44, O125, and O128 (3.92%); O78 (1.96%); and untyped *E. coli* (7.84%). There was a significant difference in the number of *E. coli* serotypes in fecal samples ($P < 0.01$). Our findings are similar to previous data that found the most common *E. coli* serotypes isolated from diarrheic fecal samples to be O119, O111, O126, and O78 (Tamaki, et al. 2005). Similarly, Badouei *et al.* isolated O157:H7, O111, and O26 serotypes from 297 fecal samples from 200 diarrheic and 97 non-diarrheic calves. The most common serogroup was O26 (18.4%).

Antimicrobial sensitivity tests showed that *E. coli* present in fecal samples were differentially sensitive to antimicrobial agents (Table 7). *E. coli* present in fecal samples were most sensitive to marbofloxacin (96.07%), enrofloxacin (88.23%), cefotaxime (84.31%), amoxicillin (78.43%), and spectinomycin (33.33%). The *E. coli* present in fecal samples were most resistant to penicillin, neomycin, erythromycin, streptomycin, tetracycline, and chloramphenicol. Our results agree with those of Sadiek and Sohair (1999) who studied antibiotic

sensitivity of fecal samples from diarrheic calves and found that enrofloxacin was the antibiotic of choice for most bacterial isolates (including *E. coli*, *Salmonella* species, *Klebsiella* species, and *Proteus* species). In another study, *in vitro* sensitivity testing of isolated bacteria from the feces of diarrheic calves showed that ciprofloxacin, enrofloxacin, and gentamicin were the most effective drugs (El-Gaml *et al.,* 2001). Recent work has also shown that ceftiofur and enrofloxacin were highly efficient antibiotics in treatment of neonatal calf diarrhea, as indicated by antimicrobial sensitivity tests (Aba-Alkhalil and El-Naenaeey, 2003). In addition, most *E. coli* isolates from diarrheic calves have been shown to be resistant to kanamycin, gentamycin, chloramphenicol, tetracycline, and ampicillin (Sato *et al.,* 2005).

To assess the influence of probiotic supplementation on diarrhea in buffalo calves, we divided the 51 diarrheic calves into 2 groups: an unsupplemented group of 26 calves was treated with Enroflox 10%, oral rehydration therapy, Finadyne, fluid therapy, and milk intake reduction for up to 24 hours until clinical improvement; and a group of 25 calves was treated in the same way, but with Lacteol Fort supplementation. These lines of treatment for calf diarrhea have been previously described (El-Sawalhy, 1999, Radostits *et al.,* 2007 and Mahmoud, 2009).

Table 8 reveal the efficacy of Lacteol Fort (*lactobacillus* culture) in increasing cure rate and reducing recurrence rate of *E. coli* diarrhea. The cure rate in the Lacteol Fort group was 80%, compared to the 69.23% cure rate in the unsupplemented group. Importantly, the recurrence rate (defined as recurrence of diarrhea within 15 days post treatment) in treated calves was 8%, compared with 26.92% in the unsupplemented group. Differences in cure rate and recurrence rate were statistically significant at $P < 0.001$. Our results concur with those of Tkalcic *et al*. (2003) who found that diarrheic calves treated with probiotics experienced mild transient diarrhea and reduced fecal shedding of *E. coli* compared to control animals.

Probiotics exert beneficial effects through various means. Probiotics create conditions unfavorable for the growth of enteropathogens, reducing the occurrence of scour and restore balance of intestinal flora (El- Sawalhy, 1999). *Lactobacillus casei* plays an important role in the prevention of enteric infection by increasing IgA secretion in the intestinal lumen, providing a defense of the mucosal surface (Predigon *et al.,* 1991 and Predigon *et al.,* 1995). Probiotics prevent colonization and overgrowth of pathogenic bacteria by adherence to the intestinal wall (Drisko *et al.,* 2003). *Lactobacillus* species can prevent the adhesion of *E. coli* and *Salmonella typhimurium* to intestinal cells and induce the secretion of anti-inflammatory cytokines (Petros *et al.,* 2006). *Lactobacillus casei* has been shown to reduce some pathological outcomes (especially diarrhea) in calves (Erickson and Hubbard, 2000). These data are consistent with our findings that *E. coli* infection is prevalent and serotypically diverse in diarrheic buffalo calves and that probiotic treatment can substantially improve recovery from diarrhea by increasing cure rates and decreasing recurrence.

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References

Aba-Alkhalil, and El-Naenaeey, E.Y. (2003): Evaluation of antimicrobial action of ceftifour sodium (Excenel), *in vitro* and *vivo*. Suez canal Vet.Med. J., VI (2):42-51.

Ashraf, N.M.R. (2007): Enzootic gram negative bacteria associated with diarrhea in neonates in Egypt. Ph.D Thesis, Dept. of Microbiol., Fac. Vet. Med., Alex. Univ.

Badouei, M.A., Salehi, T.Z., Khorasgani, M.R., Tadjbakhsh, H., Brujeni, G.N. and Nadalian, M.G. (2010): Virulence gene profiles and intimin subtypes of Shiga toxin-producing Escherichia coli isolated from healthy and diarrhoeic calves. Vet Rec. 167(22):858-61.

Boyd, J.W., Walker, J.R., and Leyland, A. (1974): Neonatal diarrhea in calves. Vet. Rec., 95(14): 310-313.

Campos, L.C., Franzolin, M.R. and Trabulsi, L.R. (2004): Diarrheogenic Escherichia coli categories among the traditional enteropathogenic *Escherichia coli* O serogroups- a review Mem. Inst. Oswaldo Cruz.; 99(6): 545-552.

Chauviere, G. (1992): Competitive exclusion of diarrheagenic Escherichia coli (ETEC) from human enterocyte-like Caco-2 cells by heat-killed *lactobacillus*. FEMS Microbiology Letters. 81:213-218.

China, B., Pirson, V. and Mainil, J. (1998): Prevalence and molecular of attaching effacing E.coli among calf populations in Belgium. 63(2-4): 249-259.

Coconnier, M.H. (1997): Antibacterial effect of the adhering human *Lactobacillus acidophilus* Strain LB. Antimicrobial Agents and Chemotherapy, 41(5): 1046-1052.

Cruickshank, R., Duguid, J.P., Marion, B.P. and Swain, R.H.A. (1975): Medical Microbiology, Vol.

2, The Practice of Medical Microbiology. 12th Ed., Churchill Livingstone, Edinburgh, London.

Drisko, A.J., Giles, C.K. and Bischoff, B.J. (2003): Probiotics in health maintenance and disease prevention. Altern. Med.Rev., 8:143-155.

El- Sawalhy, A. A. (1999): "Veterinary Infectious Diseases" 2nd Edit. Pps. 329-336. Ahram Distribution Agency, Egypt.

El-Gaml, A.M., Hashem, M.EL. and Hatab, M. (2001): Diarrhea in kids attributed to enterobacteria and cryptosporidium. Assiut Vet. Med. J. ,45(89):132-141.

Erickson, K.L. and Hubbard, N.E. (2000): Probiotic immunomodulation in health and disease. J. Nutr., 130:403s-409s.

Ewing, W.H. (1986): Edwards and Ewing's identification of Enterobacteriaceae, 4th edition, pp, 135-172. New York: Elsevier.

Farid, A., Eid, G.E., Abdel- Mawla, Y.R. and Nagat, A.S. (2001): Evaluation of the efficacy of Escherichia coli (K99) vaccine on the incidence of *Escherichia coli* and immunity in buffaloes. Vet. Med. J., Giza, 49(3): 385-399.

Gyles, ,C.L. (1993): *Escherichia coli*. In: Gyles CL, Thoen CO, eds. Pathogenesis of Bacterial Infections in Animals, 2nd ed, Ames, Iowa: Iowa State Univ Pr, 164–187.

Harbby, H.A. (2002): Bacterial causes of diarrhea in small animals (Kids, lambs and calves) in Sultanate of Oman. J. Egypt. Vet. Med. Ass., 62(3): 227-235.

Hughes, D.T., Terekhova, D.A., Liou, L., Hovde, C.J., Sahl, J.W. and Patankar, A.V. (2010): Chemical sensing in mammalian host-bacterial commensal associations. Proc Natl Acad Sci USA.107:9831–9836.

Ibrahim, E.D. (2007): Studies on microbial causes of diarrhea in calves. M.V.Sc. Thesis, Fac. Vet. Med., Kafr El-Sheikh Univ.

Koneman, E.W.; Allen, S.D.; Dowell, V.R.; Janda, W.H. and Sommers, H.M. (1992): Color atlas and Textbook of Diagnostic Microbiology. 4TH Ed., J.B. Lippincott CO., New York.

Mahmoud, A.A. (2009): Animal infectious diseases. 7th Ed. PPs. 298-302. Faculty of Veterinary Medicine, Alexandria University, Egypt.

Novert, A. and Hammad, A.(2001): Studies on mycotic and bacterial enteritis. J. Egypt. Vet. Med. Assoc, 61(6 B): 190-201.

Oxoid (1987): Agents and main distributors, the manual, 6th Ed.

Petros, A. Maragkoudakis, Gaorgia Zoumpopouleu, Christes Mlaris, Gaorge Kaalantzepoules, Brune Pet and Effie Tsakalldeu (2006): Probiotic potential of *lactobacillus* strains isolated from dairy products. Int. Dairy J. ,16 (3): 189-194.

Predigon, G., Alvares, S. and Ruzi Holgado, A. (1991): Adjuvant activity of oral *Lactobacillus casei*: influence of dose on the secretary immune response and protective capacity in intestinal infections. J. Dairy Res., 58:485-496.

Predigon, G., Alvares, S., Rachid, M., Aguero, G. and Gobbato, N. (1995): Immune system stimulation of probiotics. J. Dairy Sci., 78:1597-1606.

Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.R. (1994): Clinical Veterinary Microbiology. Mosby. Yearbook Europe Limited.

Radostits, O.M., Gay, C.C., Hinchcliff, K.W. and Constable, P.D. (2007): Veterinary Medicine, 10th edition. PP. 847-888. Saunders, Philadelphia.

Rolfe, R.D. (2000): The role of probiotic cultures in the control of gastrointestinal health. J. Nutr., 130 (2 suppl): 396s- 402s.

Sadiek, A. and Sohair, Z.H. (1999): Acute enteritis in neonatal calves in Sohag governorate with special reference to aetiology, clinico-chemical aspects and therapy. Assuit Vet. Med. J., 42(83):200-212.

Sato, K., Baretlett, P.C. and Saeed, M.A. (2005): Antimicrobial susceptibility of *E. coli* isolates from dairy farms using organic versus conventional production methods. J. Am. Med. Assoc., 226 (4): 589-594.

Tamaki, Y., Narimatsu, H., Miyazato, T., Nakasone, N., Toma, C., and Iwanaga, M., (2005): The relationship between O antigens and pathogenic genes of diarrhea- associated *E. coli.* Jpn. J. Infect. Dis., 58: 65-69.

Tkalcic, S., Zhao, T., Harmon, B.G., Doyle, M.P., Brown, C,A. and Zhao, P.(2003): Fecal shedding of enterohemorrhagic *E. coli* in weaned calves following treatmrnt with probiotic *E. coli.* J. Food Prot. 66(7): 1184-9.

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Stigma, Discrimination, and the Consequences of HIV-AIDS for People Living With It in Iran

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Abstract: There is evidence that a significant proportion of People Living with HIV (PLWH) still deal with stigma and discrimination in their everyday life. The social factors attached to HIV/AIDS are a major obstacle in the curtailment of the disease requiring urgent action. This qualitative study explored perceptions of PLWH regarding HIV/AIDS Consequences in the Islamic Republic of Iran. In-depth, semi-structured interviews were held with a purposively selected group of PLHIV recruited from three HIV care clinics in Tehran. All interviews were recorded and typed, and the codes were extracted after reviewing them several times Data were analyzed using the content analysis approach. In this study 34 participants were interviewed. Of the participants, 26 were HIV-positive, 22 of which were men and four women.. In addition, five healthcare workers and three wives of HIV-patients were interviewed. Participants were referred to the following consequences: Social, mental, physical and economic. Most of the participants named social outcome (discrimination and stigma) as the most important consequences for HIV/AIDS. The most important outcome of mentioned by the HIV-positive patients, healthy spouses, and healthcare workers was social consequence (discrimination and stigma). We need effective strategies to reduce HIV stigma as treatment and care resources are scaled up in the settings that are most heavily impacted by the HIV epidemic.
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Keywords: HIV; AIDS; Iran; Consequence; Qualitative research.

1. Introduction

Since the beginning of AIDS epidemic in 1981, some 60 million people have been infected and 25 million deaths have been caused by HIV-related symptoms (UNAIDS, 2009). Therapeutic progress has improved the health condition of HIV-AIDS patients and their longevity (Strebel, 2009; Poudel, 2007; Guimaraes, 2008; Eisele, 2009). Thus, the number of people living with HIV (PLWHs) is increasing, as well. The attitude of people toward PLWHs is different in various societies, and in many cases, it is negative and discriminative. The way people look at individuals living with the disease depends on the social acceptance of disease transmission in the society. Stigma, discrimination, and phobia are among the most common worries of healthcare workers who provide services for these patients, and the therapeutic staff sometimes avoids interacting with the patients (The World Bank Report, 2011). Fear of stigma, anxiety, disappointment, depression, stress, lower perceived quality of life are among the problems patients face after their HIV diagnosis (The World Bank Report, 2011; Maman, 2009; O'Connell, 2003).

In Iran, the first HIV case was reported in 1987, and it was caused by polluted blood products. Subsequently, more cases were identified in the country's prisons. It's believed that Iran has the most number of intravenous drug users in the Eastern Mediterranean region and North Africa, and the AIDS epidemic is concentrated in this group. According to a 2007 estimate, 14 percent of intravenous drug users in the country are infected by HIV (UNAIDS, 2011). Currently, the low-level epidemic in Iran has increased to a concentrated epidemic. Studies performed by WHO indicate an estimated 80,000 infected individuals in Iran but just a one-fourth, 23,125, of patients have been identified as of June 2011. Among the infected, 91.5 percent are men and 8.5 percent are women. It was reported that in 69.8 percent of cases, the virus was transmitted through infected needles and syringes for injection, and 10 percent through sexual relationship. In 18.3 percent of cases, the cause of transmission is not clear (Center for Disease Control, 2011). Although the percentage of sexual transmission among the identified cases has remained constant during recent years, its absolute number has been

increasing continuously. The percentage of unknown transmission has gone up as well during recent years, at least in part related to the increase in sexual transmission, which usually remains unknown because of the stigma associated with this disease (National AIDS Committee Secretariat, 2010). This stigma can also affect preventative actions, such as using condoms, testing, and measures to prevent the vertical transmission of disease from mother to child (Maman, 2009). Therefore, special attention should be paid to prevention plans due to the sexual transmission of HIV in the country.

Investigation of perceived experiences of PLWHs about the consequences of being infected by this disease and the derived stigma and discrimination can improve prevention and social behaviors among this group. On the other hand, planning to preserve the social rights of PLWHs can improve the quality of their lives by reducing this stigma and discrimination. Since qualitative research can help us to understand what people believe and their real lives (Gerish, 2006), the present study is conducted with a qualitative approach aiming to investigate the attitude of PLWHs toward the consequences of AIDS affliction, and its results can be applied to improve the quality of their lives, encourage voluntary disclosure of their disease while using therapeutic services, and persuade them to consider prophylactic sexual behavior.

2. Material and Methods

To investigate the attitude of people with HIV-AIDS toward the consequences of being afflicted, qualitative research has been applied in the present study. In 2010, the research was performed during nine months in three HIV care clinics (consulting clinics of behavior disorder) in Tehran. Two clinics were attached to Shahid Beheshti University of Medical Sciences and Health Services (Valfajr and 12th of Farvardin) and the other one (Consulting Clinic of Imam Khomeini Hospital) is attached to Tehran University of medical Sciences. The Consulting Clinic of behavioral disorders of Imam Khomeini Hospital is one of the biggest centers that provides services for people living with HIV and makes it possible for patients to use professional medical services as it is located near Imam Khomeini Hospital. The studied sample in this research includes people with HIV-AIDS. Considering the limited access to patients and the limitations of investigation, the convenience sampling was applied and the referees (PLWHs) to these clinics were included. At last, it was decided to apply the theoretical sampling with maximum variation and those with different viewpoints toward the issue were chosen. The most possible variation by age, marital status, education, and employment was attempted. The interviews continued until data saturation was reached. The questions included were deep and semi-structured. Having the prior permission of interviewees, after recording their demographic information on paper, a tape recorder was used to record the interview. All of the interviews were transcribed and typed at the earliest convenience. Then, the data was analyzed by content analysis. Firstly, the interviews were reviewed several times and a perfect acquaintance was achieved, then the codes were extracted and categorized and the main themes were specified. After coding each interview, the procedure was performed for the next one.

To confirm the credibility of qualitative data, some of the coded interviews were returned to the participants to be reviewed by them to find if the interspersion of results is in accordance with their attitude or not. The long-term involvement of researchers with participants and the devotion of adequate time by them also confirm the creditability of this study. The external check applying the complementary comments of coworkers by separated coding of half of the interviews was performed as well. The triangulation through reconciliation of data collecting methods (interviewing the patients and revision of their cases), reference reconciliation of data (interviewing male and female patients, therapeutic staff, non-infected wives of patients), choosing the participants from different consulting clinics of behavior disorder, and performing a maximum variation sampling, were all attempted in this research, which increases its creditability. Dependability of achievements was evaluated by external check. To investigate any possible incoherence in coding, the transcripts of several interviews were given to another researcher to be coded separately. The incoherent achievements were investigated to evaluate their validity and the causes were identified. The opinions of qualitative researchers were applied as well. The research methodology was described in detail to confirm its transferability.

To start the task in consulting clinics of behavior disorder, we first got the approval of assistants of Tehran and Shahid Beheshti University of Medical Sciences and Health Services. At the beginning of the interview, the aims of the research were described, and the interviewees were asked if they were ready for the interview. The interview was not performed in cases of discomfort. The participants had the right to end the interview in any stage. The verbal and conscious acquiescence of interviewees was taken for participation in the research and recording their interview. The protection

of privacy due to personal information was considered at all stages. The interview was performed in separate rooms in absence of therapeutic staff and the possibility of other patients passing. The duration of interviews ranged from 41 to 90 minutes, depending on their process. The interviews continued until data saturation was reached.

To achieve a wider range of opinions, the interview was also performed for five employees of consulting clinics of behavior disorder (including doctor, psychologist, social worker, and consultant) and they mentioned the consequences for their patients that they had found out during visiting and consulting their patients. Three negative HIV women with infected husbands were also interviewed and they expressed the consequences from the viewpoint of their husbands. In four cases, the interview was performed in two sessions. In this research, three people among those asked were not willing to be interviewed.

3. Results

In this study, 34 people were interviewed, 26 of whom were infected by HIV-AIDS. Twenty-two patients were men with an average age of 37.5 and a standard deviation of 7.13. The minimum age of men was 27 and the maximum was 53. Four patients were women whose average age was 34 with a standard deviation of 3.04. The minimum age of women was 30 and the maximum was 38. The diagnosis duration for men was about five years and for women it was 7.5 years. Due to the stage of disease, in 64 percent of men and 50 percent of women it had progressed to AIDS. The demographic and behavioral characteristics of patients can be observed in table 1.

Table 1: Demographic and behavioral characteristics of the studied HIV patients

variant	Men (22 individuals)	Women (4 individuals)
age	19-29 years (3) 30-39 years (11) 20-49 years (6) Elder than 50 (2)	30-39 years (4)
Marital status	Single (11) Married (9) Divorced (2)	Married (1) Divorced (1) Widowed (2)
employment	Unemployed (8) Employed (13) Retired (1)	Housewife (unemployed) (2) Employed (2)
Education	Uneducated (2) Elementary (3) Lower secondary (6) Upper secondary- diploma (10) Further education (1)	Lower secondary- orientation cycle (2) Upper secondary- diploma (2)
Imprisonment record	Positive(19) Negative (3)	Positive (0) Negative (4)
Sexual contact	Have used condom in last sexual contact (11) Had non in last year (9) Haven't have used condom in last sexual contact (2)	Have used condom in last sexual contact (1) Had non in last year (3)
Previous drug addiction	Methanol Maintenance Treatment (9) Narcotics Anonymous Recovery (11) No addiction record (2)	No addiction record (4)
High risk behavior	Intravenous drug addiction (10) Unprotected sexual relationship (2) Intravenous drug addiction & unprotected sexual Relationship (10)	Sexual relationship with infected Husbands (4)

Table 2: The issued themes and subthemes about the consequences of HIV-AIDS affliction

themes	subthemes
Social consequences	1. stigma and discrimination on people with HIV-AIDS and their excommunication from the society, entourage and family 2. limitation due to the social activities and employment 3. limitation in starting family and protecting it and the impossibility of having a child 4. the stigmatization of patients by therapeutic staff which limits them in receiving therapeutic services
psychological consequences	1. Chronic stress and anxiety before the certain diagnosis of disease 2. depression and seclusion after the certain diagnosis of disease 3. the constant worry due to the future and how long they would live 4. desire for death, thinking about suicide and proceeding to its committal 5. the effects of thinking about the disease on their daily lives and nightmares
Physical consequences	1. losing weight, physical abilities and disability in doing their jobs and activities 2. the consequences of taking Antiretroviral drugs
Economical consequences	1. spending time for diagnostic and therapeutic tasks 2. spending money (financial cost) for therapy 3. unemployment derived by their affliction

Interviewees' answers to questions such as "what problems were raised by the affliction for the infected people?"; "what problems you have been through because of this disease and how much do you think this disease has affected you?"; "what kind of disease do you think the AIDS is?" helped us to investigate their attitude toward perceived severity of HIV-AIDS after being infected.

The mentioned consequences after HIV infection are categorized in four main themes including social, mental, physical, and economic consequences. The themes and subthemes issued by the interviewees are observable in table 2.

3.1 Social Consequences

According to the majority of patients, therapeutic staff and the healthy women with infected husbands, the social consequences are the most significant ones and many of them consider it even more important than physical consequences and economic problems caused by the disease. Among the issued subthemes in theme of social consequences, the stigma and discrimination are given higher priorities. According to almost all of them, the stigma and discrimination on PLWHs and their excommunication from society, relatives, and family are the most significant consequences of HIV in Iran. Regarding the excommunication of infected people a 40-year-old married man said: "We made a mistake by telling everyone about our problem. First we told my mother, my mother told my sister, and she announced it to all relatives. From then on, we were embarrassed wherever we went. We are now absolutely rejected. We have moved house to the middle of desert. We go to no one's house and we don't let anyone comes over."

About the existing stigma among the society even the therapeutic staff, toward the patients and female ones in particular, a 30- year old infected woman said: "My leg was wounded. The doctor wanted to touch it when I told him not to. I'm HIV-positive. His secretary said: 'are you HIV positive? How did you get it! You don't seem to ...' if they understand that a woman is infected they would immediately think that she must have been infected through adultery."

A 37-year-old single man described the negative attitude of society toward the patients: "If they do not have a set of information, they would look at you as if they are looking at a mare microbe or an injection!" The doctor of the consulting clinic, who is in charge of visiting the patients and consulting with them, believes that: "AIDS is like cancer. Everyone looks for cancer patients to express their sympathy, but as to AIDS patients, they are all abandoned because of the stigma against them."

More than half of the individuals mentioned the limitation due to the employment, having social activities, and the impossibility of disclosure of their disease in workplace. A 34-year-old single man said: "Someone found me a job not long ago to go and work in the kitchen of a company, they would even insure me. I had to lie to avoid accepting this job, for which I was blamed too much. I was told that I wasn't diligent; otherwise I wouldn't miss such a good job."

The existing limitation to start a family is another social consequence mentioned by the majority of patients and the non-married ones in particular. A 41-year-old single man said: "Since the beginning of this year, I have decided to get married, but I still haven't found a good case. I think you should spend time on the marriage of HIV-positives. Consider me as well. We are so lonely." Most of the single patients and the married ones who have no children mentioned having children as one of their distresses. A 33-year-old divorced woman said: "I wish to have a child and take him (her) to school. I wish to see him (her) until the age of 7 or 8. It's the only wish I have, and then I would die." Another social consequence mentioned by the majority of patients is the lack of receiving therapeutic services after revealing their disease to the therapeutic staff. A 36-year-old married man said: "I have seen this bad reaction even by the doctors. Right now, I myself have a hernia. Where ever I go for the surgery, they avoid it after finding out that I'm HIV-positive. I remember when I went to hospital for a lung surgery several years ago. The doctor forced me out of his room with a harsh reaction as soon as he learned about my disease."

3.2 Psychological Consequences

All the patients mentioned that they have been dealing with psychological complication after becoming aware of their affliction. They pointed out their chronic anxiety and stress before the diagnosis of the disease and the lack of psychological comfort in their lives. A 38-year-old married man described his anxiety before HIV diagnosis: "The doctor said: we guise it should be this disease, but it takes 10 days to answer you certainly. During those 10 years I had too much stress which caused me to loose some 10 kilograms. I caught shingles. The doctor said it's caused by chronic stress."

Most of the patients mentioned depression as the most important psychological consequence of HIV affliction. A 38-year-old widow said:

"There is stage for all who first understand that they are HIV-positive, which really cause them a lot of psychological difficulty. I myself have passed a six-month deep depression. I wanted to see no one

and go nowhere. I was fighting with God asking why me. I couldn't understand why I should be in such pain."

Less than half of the patients mentioned their worry for the future considering their disease. A 51-year-old married man said: "Is there any idea about me and the future of my disease? ... Have anybody thought about my AIDS, or they are merely waiting for me to die and become eliminated?"

Another psychological consequence mentioned by fewer patients was the desire for death and suicidal thoughts.. A 53-year-old married man mentioned: "Sometimes I touch the electrical wire. I'd really like to kill myself if it wasn't a sin. The improper reactions of others I went to do it several times. The wire was in this hand, but I didn't have the power to put it on the other one." The effect of thinking about the disease on the daily life and the nightmares was expressed by a 35-year-old single man: "... I think that I'm dying right now, I smell death. I don't know if you have ever gone to a cemetery. Have you smelled its soil? When I sleep, there are nightmares, when I get up, I smell death."

3.3.Physical consequences

AIDS affliction causes many physical problems for patients. The majority of patients, especially those in AIDS stage, pointed out weight loss, reduction of physical abilities, and disability due as the physical consequences of their affliction. A 36-year-old single man said: After AIDS affliction, one becomes tired and weak. He doesn't have the power to do his job. I don't have the energy to work. After two months, I found that I can't work eight hours a day anymore."

The symptoms caused by adverse effects and interactions of the drugs taken, was mentioned by the majority of patients and in particular, those taking Anti Retro Viral in AIDS stage. A 44-year-old man described the adverse effects of Anti Retro Viral: "When I start taking pills I couldn't walk more than three steps. I had pain in my heart and chest, I had tremendous headaches and my eyes were puffy. I had a bone marrow biopsy and they told me that these are caused by one of the drugs. Things were fixed when I stopped taking that one." A 35-year-old single man under methanol maintenance treatment described the drug interaction: "The drugs have inactivated the effect of methadone. When I take my AIDS drug, my head tumbles spontaneously while having food. Everyone think that I'm slumbering. My spoon suddenly falls which is because of the adverse effects of drugs on me and the methadone I take. After 8 o clock, I have such a terrible situation that my mother cries with me."

3.4. Economic Consequences

Some of the patients mentioned economic problems as the consequences of AIDS-affliction and they said that such problems are caused by spending time and money for therapy and unemployment resulting from their HIV-affliction. A 36-year-old married man said: "During several recent years, this disease has caused me to lose a lot of time. If I weren't HIV-positive, I would be in a better economic situation. On the other hand, I should spend a lot on my therapy. I should eat more nutrient food and take better drugs and vitamins, so I should spend more on them. I should come to hospital every week which would cost me a lot of carfare."

3.5 Disease description

The description of AIDS by patients was either in negative or positive forms. The majority of them described the disease negatively using terms such as: "terrible disease, incurable, suffering, and high-risk disease, a tragedy, assuming them dead, from the moment they got the disease, a parameter which results in loosing life, a disease which devastates all one's golden years." Just two patients thought that their affliction had some positive effects. They believed that HIV-affliction had improved their self-confidence and ability.

4. Discussions

The AIDS epidemic has caused people to have some negative reactions, including prohibiting HIV-positives to come to their houses and isolation from family, their dismissal from workplace, and refusing to accept infected children in schools. The negative reactions create behaviors in infected individuals, which limit the efficiency of preventing activities such as using condoms and plans to prevent vertical transmission of disease from mother to child and doing HIV tests. AIDS causes anxiety as death comes along with it. HIV-infected individuals receive less social support compared to cancer patients (Mawar, 2005). PLWHs would face many problems after their affliction and they are exposed to the risk of psychological problems such as depression and anxiety. Having the experience of an acute illness, the change in employment situation, and the adaption to complicated drug diets are all continual sources of stress for the infected individuals. Medicinal treatments can have chronic adverse effects. Apart from such effects, taking drugs is a daily reminder that the person is ill. This can also be included as a source of stress for the patient. In many cases, the HIV diagnosis would cause a certain discrimination and excommunication for the infected person. Despite some successful treatments, the early death

of patients caused by high-risk behaviors and suicide is still possible (Scheid, 2008).

In the present study, the majority of patients, therapeutic staff, and even the wives of patients believed that the social consequences are the most important ones caused by HIV-affliction. According to all of them, among the social consequences, the stigma and discrimination toward patients and their excommunication from society, relatives, and family are the most significant problems. The limitation in social activity and employment, starting a family and protecting it, the impossibility of having children, and the stigmatization of patients by therapeutic staff, which limits them in receiving therapeutic services, are the social consequences mentioned in lower levels of significance. PLWHs would be encouraged to reveal their disease to their families voluntarily, as they need the emotional and family support especially to start Anti Retro Viral Therapy. Disease disclosure would accelerate the discriminative behaviors of family members (Mills, 2009). Stigma seems to be a key stop that decreases the quality of therapeutic care (Chan, 2008). Many studies on the stigma and discrimination against HIV-patients have been performed in Iran. According to the survey performed by Montazeri, the achievements indicate that although there is some wrong perceptions about AIDS in Iran, by and large a rather good knowledge and positive attitude toward the disease and the patients is prevalent among healthy individuals of the society (Montazeri, 2005). According to Cao et al., 80 percent of studied people in China were afraid of PLWHs and this negative attitude was directly related to their lower levels of awareness and education, and older ages (Cao, 2010).

According to the study by Kabbash, et al., in Egypt, the majority of infected people were dealing with anxiety, hopelessness, depression, anger, and feelings of disability and helplessness. Half of them mentioned the stigma they feel from the other members of society. Being afraid of such stigma has caused two thirds of them to isolate themselves from society. Half of them had a feeling of uselessness in their society. The patients mentioned that they need a social support for they are afraid of revealing their disease, pain, the future of themselves and their families, and death. The change in their employment situation, inability to perform their job and the same activities they used to do before their affliction, the lack of facilities for job promotions and the opportunities for leisure activities, were also indicated by the patients. The patients experienced economic difficulties and they were afraid because they thought it would prevent them from having nutritious food and using adequate care services in future. Although some of the patients were upset about the negative attitude of therapeutic staff toward them after knowing about their disease, most of them mentioned that they have been truly supported by healthcare workers of AIDS programs, especially when needing vital care such as surgery. The need to have a life without stigma, fear, or stress was also mentioned by the patients. Most of them said that they have had to change their sexual behavior pattern (Kabbash, 2008).

According to the qualitative study of Rahmati, et al., on HIV patients in Iran, almost all of the patients have felt stigmatized and discriminated by healthcare workers. As a consequence of such stigma and discrimination, the patients would postpone or avoid receiving therapeutic services or revealing their disease to therapeutic staff, their motivation to protect their health would decrease, they would feel a sense of malice or vengeance, they might use alternative medicine or they would feel emotionally stressed (Rahmati, 2010). In the study by Xianhong, et al., more than half of the PLWHs had experienced the stigma. Social excommunication and financial insecurity reflected the external stigma and 84 percent of them have experienced financial problems. The internal stigma included negative self-worth, interpersonal security, and the anxiety for disease disclosure, respectively reported by 78 percent, 75 percent, and 58 percent of individuals (Xianhong, 2009). The study performed by Liamputtong, et al., on HIV infected women in Thailand, indicated that the beginning of AIDS-related physical changes and symptoms in their body would cause more stigmas against them. For such changes represent an imminent death. Losing the job is another consequence of HIV affliction (Liamputtong, 2009).

The studies indicate that AIDS-related stigmatization against the patients by health workers is usually caused by their lack of awareness, fear of having accidental contact with the patients, and association of the AIDS patient with immoral behaviors. Thus, to remove such stigma, interventions should be performed in three individual, environmental, and policy levels (Nyblade, 2009). The results of other studies also indicate that a part of such stigmatization and discrimination by family, society, and therapeutic staff can be related to their unawareness of methods of transmission of the disease or unreasonable fear. By improving the level of awareness, particularly through public media, besides the obviation of the AIDS obscenity, the social excommunication, stigma, and discrimination toward PLWHs can be prevented as well. The derived psychological effects in patients not only go back to their beliefs about incurability of their disease, but they can also be related to the AIDS

obscenity in Iran caused by the negative attitude of people toward AIDS as a sexually transmitted disease. Improving the public awareness and the services provided can remove the psychological consequences and promote the life quality of PLWHs. On the other hand, it would prevent further transmission of the disease among families, health care centers, and sexual partners because of non-disclosure of disease. Losing the job and the limitation of insurance coverage affects the utilization of patients from therapeutic services. Despite the fact that in Iran, many outpatient therapeutic and in vitro services are free for this group of patients, because of the increase in their longevity and the more need to professional and clinical therapeutic services, it is required to widen the insurance coverage and upgrade the level of commitment because of these patients. As was clear in other studies as well, the sense of belonging to a supportive AIDS group helps the patients to counter the stigma and discrimination they feel in society (Liamputtong, 2009). Therefore, supporting those in charge of supportive groups for PLWHs can help them remove such stigmas and discriminations from the patients.

Based on the achievements of the present paper, it is suggested that to remove such stigma and discrimination from the patients, the awareness of society and therapeutic staff has to be increased, which requires broadcasting continual and public training. On the other hand, it is also suggested to encourage and support the ones in charge of forming AIDS support groups to help them to remove the stigma and discrimination from the patients. Finally, the public and general insurance coverage affect the better utilization of infected patients from professional therapeutic services, which is possible through some changes in policy and planning by those in charge.

Despite all efforts to increase trustworthiness and rigor of this research, it still has some limitations which are observable in all qualitative studies. One of these limitations is that unlike quantitative studies, the sample volume is usually small in qualitative ones and it might not be that much comparative and distributable to society (Adib Hajbagheri, 2007). In this study, just the attitudes of referee PLWHs to consulting clinics of behavior disorders were investigated, and the achieved results might be rather different with results related to those who don't come to such clinics. Not telling the truth is another limitation related to cultural-social problems due to people's self report, especially as the individual would be in a face-to-face situation with interviewer while being interviewed for a qualitative research. In this study, the researchers attempted to gain the maximum trust of interviewees by establishing appropriate relationships and showing the interest of researcher in statements of participants. Thus, it is necessary to consider these limitations during result interpretation.

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References

1. Adib Hajbagheri M., Parvizi S., salsali M.Qualitative research.1st edition. Boshra Publication, 2007. (Persian). 31-33.
2. Cao. H, He N., Jiang Q., Yang M., Liu Z., Gao M., et al. Stigma against HIV-infected persons among migrant women living in Shanghal, CHINA. AIDS Education Prevention 2010. 22(5): 445–454.
3. Center for Disease Control, Office of the Deputy for Public Health, Ministry of Health and Medical Education of the IR Iran. HIV/AIDS in Iran (Cumulative Statistics). Tehran: Ministry of Health and Medical Education of the IR Iran; 2011. http://port.health.gov.ir/mfdc.cdc. Accessed on 26th September , 2011.
4. Chan K.Y., Stoové M.A, Reidpath D.D. Stigma, social reciprocity and exclusion of HIV/AIDS patients with illicit drug histories: A study of Thai nurses' attitudes. Harm Reduction Journal 2008; 5(8):1-11.
5. Eisele T. P., Mathews C., Chopra M., Lurie M.N., Brown L., Dewing S., et al.Changes in risk behavior among HIV-Positive patients during their first year of antiretroviral therapy in Cape Town South Africa. AIDS and Behavior 2009; 13(6):1097–1105.
6. Gerish K., Lacey A .The Research Process in Nursing. 5th edition. Oxford. Blackwell publishing. 2006. 337-339.
7. Guimaraes M. D .C. , Grinsztejn B., Chin-Hong P.v., Campos L. N., Gomes V.R., Melo V.H., et al. Behavior surveillance: Prevalence and factors associated with high-risk sexual behavior among HIV-infected men in Brazil in the post-HAART era. AIDS and Behavior 2008; 12(5):741–747.

8. Kabbash I.A., El-Gueneidy M., Sharaf, A.Y., N.M. Hassan and A.N. Al-Nawawy (2008). Needs assessment and coping strategies of persons infected with HIV in Egypt. Eastern Mediterranean Health Journal 2008 ;14 (6):1-13.
9. Liamputtong P., Haritavorn N., Kiatying-Angsulee N. HIV and AIDS, stigma and AIDS support groups: Perspectives from women living with HIV and AIDS in central Thailand. Social Science & Medicine 2009; 69 : 862–868.
10. Maman S., Abler L., Parker L., Lane T. , Chirowodza A., Ntogwisangu J., et al. A comparison of HIV stigma and discrimination in five international sites: The influence of care and treatment resources in high prevalence settings. Social Science & Medicine 2009; 68: 2271–2278.
11. Mawar N., Sahay S., Pandit A , Mahajan, U.The third phase of HIV pandemic: Social consequences of HIV/AIDS stigma & discrimination & future needs. Indian Journal of Medical Research 2005. 122:471-484.
12. Mills E.& Maughan-Brown B. Ties that Bind: HIV-Disclosure as Consequence and Catalyst of Stigma and Support in Households . CSSR Working Paper 2009; No. 266 .December 2009. 11-12.
13. Montazeri A .AIDS knowledge and attitudes in Iran: results from a population-based survey in Tehran. Patient Education and Counseling 2005; 57 : 199–203.
14. National AIDS Committee Secretariat, Ministry of Health and Medical Education. On Monitoring of the United Nations General Assembly Special Session on HIV and AIDS. (Iran UNGASS Report) 2010. http://www.unaids.org/en/dataanalysis/monitoringcountryprogress/2010progressreportssubmittedbycountries/iran-2010-country-progress-report. Accessed on 9th April, 2011.
15. Nyblade L., Stangl A., Weiss E., Ashburn K.Combating HIV stigma in health care settings: what works? Journal of the International AIDS Society 2009; 12(15):1-7.
16. O'Connell K. Preliminary development of the World Health Organsiation,s Quality of Life HIV instrument (WHOQOL-HIV):analysis of the pilot version. Social Science & Medicine 2003;57: 1259–1275.
17. Poudel K.C, Nakahara S, Poudel-Tandukar K, Jimba M. Perceptions towards preventive behaviors against HIV transmission among PLWHA in Kathmanda, Nepal. Public Health 2007;121(12): 958 – 961.
18. Rahmati-Najarkolaei F., Niknami Sh., Aminshokravi F., Bazargan M.,. Ahmadi., Hadjizadeh A., et al.Experiences of stigma in healthcare settings among adults living with HIV in the Islamic Republic of Iran. Journal of the International AIDS Society 2010; 13(27)1-11.
19. Scheid T. Mental Health Consequences of HIV/AIDS: Effects on Substance Abuse and Treatment Adherence 2008. Available online at :http://www.allacademic.com.
20. Strebel A., Cloete A., Simbayl L. Social aspect of HIV/AIDS and health four-country report on formative research into the development of a HIV behavioral risk reduction intervention for PLWHA Botswana, Lesotho, South Africa and Swaziland. HSRC (Human Sciences Research Council 2009). Available online at: http://www.hsrc.ac.za/HAST-Projects.phtml. Accessed on 9th Jun, 2011.
21. The World Bank Report. Characterizing the HIV/AIDS Epidemic in the Middle East and North Africa: time for strategic action. 2010. Available online at: http://issuu.com/world.bank.publications/docs/9780821381373. Accessed on 28th May, 2011.
22. UNAIDS. AIDS Epidemic Global Facts and Figures Factsheet. Available online at: http://www.unaids.org/en/media/unaids/contentassets/dataimport/pub/factsheet/2009/20091124_fs_global_en.pdf. Accessed on 9th May, 2011.
23. UNAIDS. Global report UNAIDS report on the global AIDS epidemic | 2010. http://www.unaids.org/globalreport/. Accessed on 22th September , 2011.
24. Xianhong Li, Honghong W., Williams M., Guoping H. Stigma Reported by People Living With HIV in South Central China. JOURNAL OF THE ASSOCIATION OF NURSES IN AIDS CARE 2009; 20(1) 22-30.

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Numerical simulation of turbulent flow in channels with three-dimensional blocks

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Abstract: Turbulent flow over surfaces roughened by simple geometric elements, such as discrete three-dimensional pro turbulences, continue to be of interest in fluid engineering from several perspectives, regular roughness elements are routinely used for heat transfer enhancement. They are also used to study surface roughness effects, in general, as they easily reproduced in the laboratory and modeled in numerical experiments. The characteristics of a turbulent flow in channels with three-dimensional blocks are investigation in the context of surface roughness effects. Reynolds-averaged, Navier-Stokes equations, coupled with the κ- ω turbulence model with near wall treatment are solved by a finite-volume method. The space-averaged velocity profile exhibits a logarithmic region, with a roughness function that varies logarithmically with the roughness Reynolds numbers. At sufficiently large distance from the roughness elements, the effect of the individual elements vanishes and the net effect on the velocity profile is felt as reduction in the constant, β, known as the roughness function, depends not only on the roughness size but also on its geometry. The different block arrangements exhibit quite distinct flow characteristics but the differences tend to vanish as the block height decreases. In general, Reynolds-averaged numerical model successfully describes the principal features of wall roughness that have hitherto for been the purr view of experimental correlations.
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Key words: Distributed roughness, Similarity law, RANS equation, κ- ω model

1. Introduction

Turbulent flow over surfaces roughened by simple geometric elements, such as three-dimensional protuberances, continues to be of interest in fluids engineering from several perspectives [1]. Regular roughness elements are routinely used for heat transfer enhancement [2], [3]. They are also easily reproduced in the laboratory and modeled in numerical experiments. Of particular concern in practical engineering applications is the existence of similarity laws, principal among which is the logarithmic velocity distribution, upon which friction factor and heat transfer correlations are based.

At sufficiently large distance from the roughness elements, the effect of the individual elements vanishes and the net effect on the velocity profile is felt as reduction in the constant, β, in the logarithmic law. This change in the constant, β, known as the roughness function, depends not only on the roughness size but also on its geometry [1], [4].

The importance of similarity laws in engineering correlations of friction and heat transfer mentioned above, and the success of these recent numerical studies suggest that a more comprehensive numerical investigation would yield useful insights into the effects of discrete roughness elements on these parameters. As neither LES nor DNS are cost effective for such purposes, here we use a numerical model based on the Reynolds-Averaged Navier-Stokes (RANS) equations and an established two-equation turbulence model to study the effect of different types of discrete roughness on the velocity profile and related correlations. In particular, the existence of a logarithmic layer is examined and the relevant parameters are identified. Although the RANS approach is not suitable for capturing the flow unsteadiness due to large-eddy motions, it is quite adequate for the present study in which only the mean quantities, both in time and space, are of interest. Calculations are carried out for three-dimensional roughness elements. A brief description of the numerical model is presented as the various components are quite well established, and a simple validation study is performed before describing the principal results.

2. Numerical model and validation

2.1 Governing equations

For steady incompressible turbulent flow, the Reynolds averaged equations for conservation of mass and momentum may be written as follows:

Continuity: $\frac{\partial u}{\partial x} = 0$ (1)

Momentum:

$$\frac{\partial}{\partial x}\left[uu-(v+v_t)\frac{\partial u}{\partial x}\right]=-\frac{1}{\rho}\frac{\partial P}{\partial x}+\frac{\partial}{\partial x}\left[v_t\frac{\partial u}{\partial x}\right] \quad (2)$$

where u is the velocity component x-direction, P is pressure, v is kinematic viscosity and v_t is the eddy viscosity obtained from the turbulence model.

Among several variations of widely used two-equation turbulence models, the low Reynolds-number (near-wall) properly resolve the complex flow behind around the ribs, following the work of [6]. This particular model is chosen because of its proven robustness and unambiguous near-wall treatment, two essential attributes in numerical modeling of separated flow about distributed ribs. The eddy viscosity is determined from two transport equations:

Turbulence kinetic energy (k):

$$(3)\ \frac{\partial}{\partial x}\left[uk-\left(v+\frac{v_t}{2}\right)\frac{\partial k}{\partial x}\right]=P_k-\beta^* k\omega$$

$$\mathrm{Re}_T=\frac{k}{\omega v},\ \beta^*=\frac{9}{100}\cdot\frac{4/15+(\mathrm{Re}_T/R_\beta)^4}{1+\mathrm{Re}_T/R_\beta},\ R_\beta=8 \quad (4)$$

Specific dissipation rate(ω):

$$(5)\frac{\partial}{\partial x}\left[u\omega-\left(v+\frac{v_t}{2}\right)\frac{\partial \omega}{\partial x}\right]=\alpha\frac{\omega}{k}P_k-\beta\omega^2$$

$$\alpha^*=\frac{3/125+Re_T/R_k}{1+Re_T/R_k},\ R_k=6$$

$$\alpha=\frac{13}{25}\frac{1/9+Re_T/R_\omega}{1+Re_T/R_\omega}\frac{1}{\alpha^*},\ R_\omega=2.95,\ \beta=\frac{9}{125}$$

$$P_k=v_t\left(\frac{\partial u_i}{\partial x_j}+\frac{\partial u_j}{\partial x_i}\right)\frac{\partial u_i}{\partial x_j}$$

Where $P_k$the production of turbulence kinetic energy and the eddy viscosity is related to k and ω as:

$$(6)\quad v_t=\alpha^*\frac{k}{\omega}$$

2.2. Computational domain and boundary conditions

The no-slip condition is applied on walls of the channel (Figs. 1 and 2).for developed flow in a long channel with regularly spaced roughness elements; the flow is periodic in the stream wise direction. Then it suffices to consider a limited solution domain and impose periodic conditions at the upstream and downstream boundaries. For a channel with three-dimensional roughness, plane-of-symmetry conditions are applied at appropriately located span wise boundaries. The specific dissipation rate, ω is specified at the first grid off the solid surface and given a value $\frac{6v}{(9\Delta n^2/125)}$ where Δn denotes the normal distance from the wall [6].more details on channel geometry, grid and flow conditions are given in later sections.

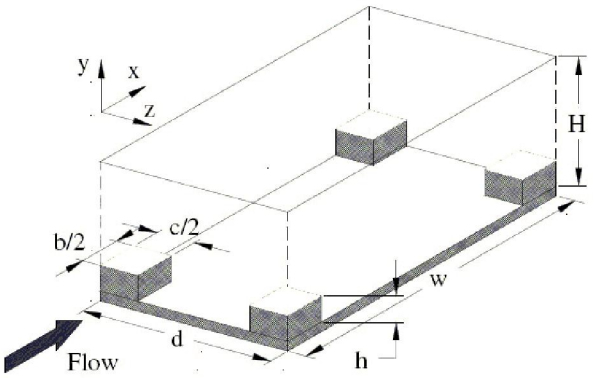

Figure.1. Schematic of channel with distributed hexahedral blocks (staggered distribution), (h = 0.1, b = 0.3, H = 0.5, d = 0.75 are fixed).

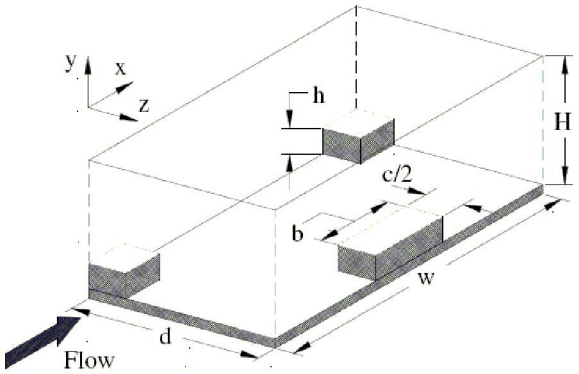

Figure.2. Schematic of channel with distributed hexahedral blocks (in-line distribution), (h = 0.1, b = 0.3, H = 0.5, d = 0.75 are fixed).

2.3 Numerical solution procedure

The above equations are solved by a finite-volume method in an orthogonal body-fitted grid.

Second-order accuracy is assured by adopting the central differencing scheme throughout except for the convective derivatives that are discretized by the QUICK scheme of [7]. The continuity and the momentum equations, and the model equations for k and ω, are solved iteratively until convergence. The convergence criterion imposed in the calculation is that the sum of the residuals of mass source be less than10^{-15}.

2.4. Validation tests

The major difficulty of model validation in the present case is the lack of adequately detailed experimental data for various rib shapes and block arrangements. In view of this, model validation is focused on two important features of the analysis: computation of separated flow and implementation of an orthogonal grid system.

The numerical model outlined above is validated against two test cases, namely, the backward facing step flow, and the square-ribbed channel flow, for which

previous calculations provide a basis for comparison. Other researchers have also used these cases to validate their solution procedures and turbulence models. Therefore, it suffices to provide a very brief description of the results.

Fig.3. Backward facing step flow for $Re_h = 2800$ and $\delta_b/h = 1.1$ at $x/h = -3.8$: (a) streamlines and pressure contours; (b) c_f distribution along the channel. For the backward facing step flow, calculations were performed in a solution domain $-3.8 < x/h < 80$ with a grid 180×80×240 , in which 30 of the 80 grid points are distributed in the expanded region, for a Reynolds number (Re_h), based on step height h and the mean inlet velocity, of 2800. The inlet velocity profile is constructed to match the turbulent boundary layer of the experiment, i.e.$\delta/h = 1.1$. Fig. 3 shows the overall flow field and the friction coefficient along the wall downstream of the step. There is good agreement with the measurements of [8] .The computed reattachment length of 6.67h. These results provide a degree of validation of the numerical method and turbulence model.

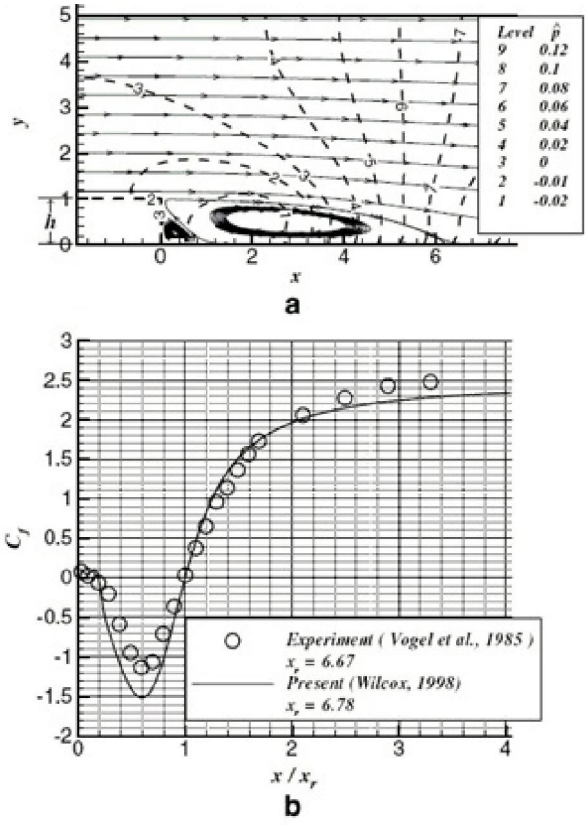

Figure.3. Backward facing step flow for Re_h=2800, (a) streamlines and pressure contours, (b)C_f distribution along the channel.

Finally , the flow over the regularly distributed square ribs with $h/D_e = 0.1$ and $w/h = 7.2$ where D_e is the hydraulic diameter, is examined for $Re_{De} = 7,200$ .The mean velocity profiles at various cross-sections plotted in Fig.4 are seen to be in good agreement with the measured data of [9].

The discrepancy observed near the top surface of the rib, where the mean velocity attains a local maximum, was first suspected to be due to the insufficient grid resolution $(100 \times 80 \times 256)$. An additional calculation with much finer grid$(160 \times 150 \times 256)$ confirms that the solution is indeed grid-independent. The two-layer model of [10] may be the only exception that qualitatively shows the local maximum in the mean velocity in that region. The results of the standard $k-\omega$ model with the wall function are shown in the figure for reference.

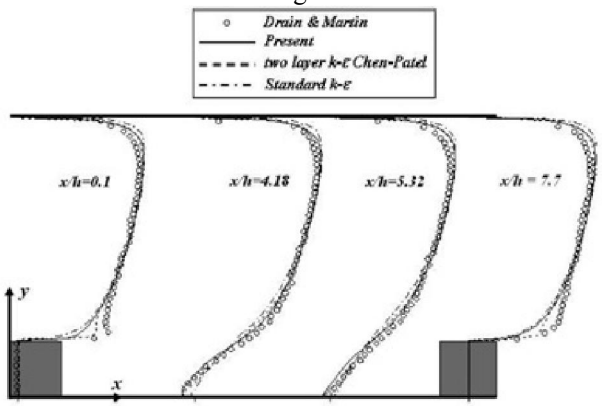

Figure.4. the mean velocity profiles at various cross-sections.

3. Results and discussion

For rough surfaces, the logarithmic velocity profile in the wall region differs from that on a smooth surface, and takes following form:

$$(7) u^+ = \frac{1}{\kappa} Ln(y^+) + B - \Delta B$$

Where $k(= 0.418)$ is the Von Karman constant, B is the smooth-wall constant$(= 5.45)$, and ΔB the roughness function, which increases with roughness size. The distance y^+ is measured from the so-called virtual origin y_0 of the wall, which also increases with increasing roughness. May be rewritten as:

$$(8) u^+ = \frac{1}{k} Ln(y^+) - R^+$$

Where:

$$(9) R^+ = \frac{1}{k} Ln(h^+) + B - \Delta B$$

Where:

$$h^+ = \frac{h u_\tau}{\upsilon} \qquad (10)$$

For sufficiently large roughness, in the so-called fully rough regime, R^+ is constant and assumes the value of 8.5 for sand-grain roughness [11].

The numerical simulations of three-dimensional flow over uniformly distributed hexahedral blocks, compared to the two-dimensional cases, there are many more geometric parameters to deal with. For present purposes, however, we examine two representative block

cross-sections: a square section and a rectangular section of spanwise aspect ratio of in-line and staggered arrangements, as shown in Fig. 1 and Fig.2. The transverse block spacing d/D_s and streamwise length of the block b/D_s are held constant at 0.75 and 0.3, respectively. The block height and pitch (streamwise spacing) are parameters that are varied. For $w/h = 15$ and 18, a non-uniformly distributed grid of 512×240×240 cells is used while a grid with 250×150×150 cells is used for $w/h = 6$, 9 and 12.

Typical flow patterns (not shown) exhibit that the flow around the staggered block is much more violent than that around the in-line blocks, in which the disturbance appears to be confined to immediate neighborhood of the block. The fluid passes between the distributed blocks in fairly orderly fashion for the in-line arrangement, while that for the staggered arrangement meanders around the obstacles and makes the vertical motion very intense. This is clearly illustrated in the cross-flow pattern of each case in Fig. 5. Fig. 5(a) shows the cross-plane streamlines at $x/D_s = 0.6$ for the staggered rectangular blocks. Two large and strong streamwise vortices span the entire channel. For the other three cases, on the other hand, the vertical motion is not as intense and is confined to the vicinity of the wall. This is true even for the rectangular block if the distribution is in-line and the square blocks in the staggered arrangement.

As is in two-dimensional case, the skin friction and pressure distributions are integrated over appropriate surface areas to calculate the effective total resistance:

$$(11)\tau_x = \frac{1}{wd}\left[\int_{s_x}(P_w - P_{ref})ds + \int_{s_y}\tau_{wx}ds\right]$$

Where τ_{wx} is the skin-friction component in the x-direction, s_x denotes the vertical surfaces of the block whose normal is in x-direction, and s_y denotes the floor and horizontal surfaces of the blocks whose normal is in y-direction. The friction factor and friction velocity are then defined as:

$$(12)f = \frac{8\tau_x}{\rho U^2} \; ; \; u_\tau = \sqrt{\tau_x/\rho}$$

Where $\overline{U}$ is the average velocity obtained by integration of the velocity profile from the lower wall to the velocity maximum at $y = \delta$.

The numerical model was again used to simulate the flow in channels with different block arrangement, varying the block height and the Reynolds number.

Fig. 6 shows the space-averaged velocity profiles in wall coordinates for **c = 2b** for three block heights and two Reynolds numbers. From these and similar plots for other geometries it is found that, in general, the velocity profiles have logarithmic regions for blocks in the in-line arrangement .The logarithmic regions are better defined as the block size is reduced and/or the Reynolds number is increased, as might be expected from the extent of the disturbance introduced by the blocks. With the staggered blocks, however, the flow is disturbed so much that a well-defined logarithmic region exists only for sufficiently small block heights, typically less than 5% of the channel height. In other words, taller blocks cannot be regarded as roughness in the traditional sense, and consequently, it is not appropriate to use correlations for friction and heat transfer based on the logarithmic velocity profile. All profiles for rectangular blocks in in-line arrangement and for square blocks in either arrangement (not shown) contain a logarithmic region.

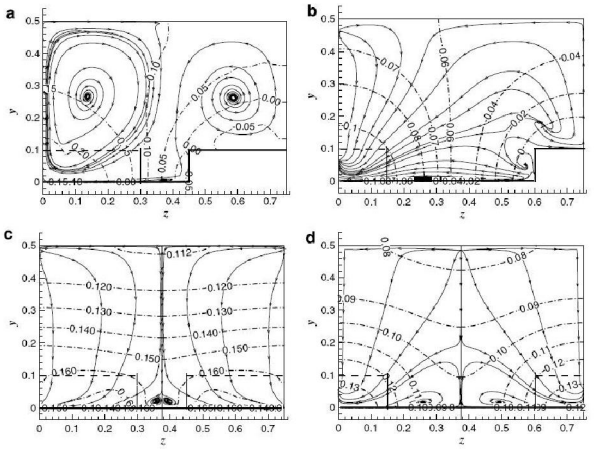

Figure.5. Cross-flow patterns and pressure ($\hat{p}$) distributions at $x/D_s = 0.6$ for $w/h = 12, h/D_s = 0.1$, and $Re_{D_s} = 20{,}000$: (a) c = 2b (staggered); (b) c = b (staggered); (c) c = 2b (in-line); (d) c = b (in-line)

As in the case of two-dimensional ribs, the resistance coefficients and roughness functions for $h/D_s = 0.1$ are shown in Fig. 7 and 8, respectively.

Some values of ΔB for this block height are estimated, as the logarithmic region could not be clearly identified for some block arrangements. The results for two-dimensional ribs of the same relative height and length to height ratio are also shown for comparison. It is seen that the friction factor and the roughness function attain maximum values somewhere between $w/h = 10$ and 15, which is very similar to two-dimensional cases. The block spacing that results in maximum drag is often associated with the point of maximum heat transfer, which is the subject of Part II of this paper. It is interesting to observe that the resistance for blocks of square cross-section is comparatively small and identical for both block arrangements while that for the rectangular block is larger and there is substantial difference between the in-line and staggered arrangements. For the rectangular blocks, the in-line arrangement gives a smaller resistance, even smaller than the two-dimensional ribs, while the staggered arrangement exerts much larger resistance on the flow than the two-dimensional ribs .this is due to the fact that the fluid has to turn more wildly to go around the

staggered blocks and, hence creates a much greater disturbance in the outer flow when the block is tall. This was confirmed by additional calculations with smaller blocks. As in two-dimensional cases, the roughness function ΔB varies logarithmically with slope $1/k$ with increasing h^+.

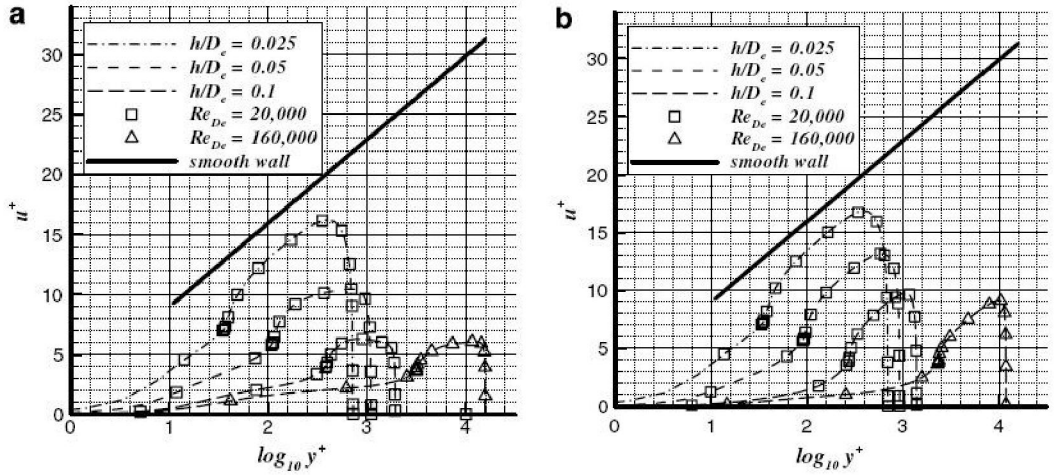

Figure.6. Mean stream wise velocity profiles in wall coordinates for $w/h = 12$ and c = 2b with various block heights: (a) staggered arrangement; (b) in-line arrangement.

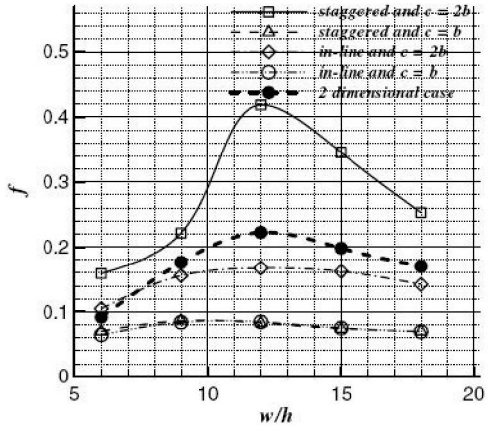

Figure.7. Friction factor vs. pitch for $h/D_e = 0.1$ at $Re_{D_e} = 20{,}000$.

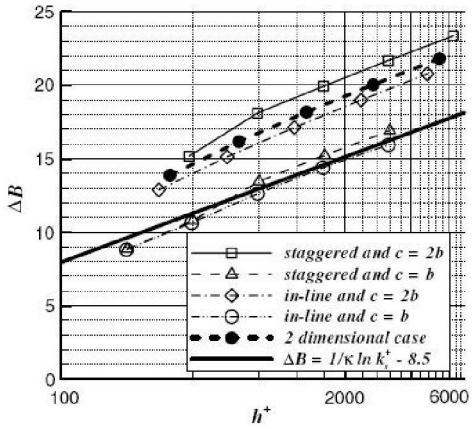

Figure.8. Roughness functions vs. pitch for $h/D_e = 0.1$ at $Re_{D_e} = 20{,}000$.

4. Conclusions

The most important conclusion drawn from this research is that a numerical model based on the Reynolds-averaged Navier-Stokes equations coupled with a turbulence model that resolves the near-wall flow is able to successfully capture the essential features of the flow over the three-dimensional blocks. When the solutions are averaged over appropriate areas, they provide engineering information about the resistance coefficient and its dependence on the geometric and flow parameters.

In addition, the numerical model provides details of the velocity profile, such as the roughness function and the virtual origin, that have hitherto for been the purview of experimental correlations, and information about the flow in the roughness layer, within the interstices of the roughness elements, that is difficult to measure and quantify by experiments. Although the latter aspects of the solutions have not been examined in any detail in the present paper, they are of interest in understanding the interaction between the spatially non-uniform flow in the roughness layer and the outer flow that feels only an averaged effect of a rough wall. Previous experience with contemporary turbulence models suggests that use of alternate models will confirm the principal results of this study.

For three-dimensional flows, the logarithmic region exists for blocks typically less than 5% of the channel height. Thus, the flow over larger blocks cannot be treated within the traditional framework of surface roughness that is based on the assumption of a logarithmic layer. Of course, this does not diminish the usefulness of the numerical model. On the contrary, a numerical model provides information that cannot be obtained by extrapolation of existing roughness correlations. The slope reaches the value of k^{-1} for sufficiently large h^+, confirming the trends established by experiment for other types of roughness. For three-dimensional blocks, the resistance and the roughness function depend on the block shape and arrangement.

References

[1] H.Schlichting, Boundary-Layer Theory, seventh ed. McGraw Hill, pp.598-634, 1979.

[2] R.L.Webb, E.R.G.Eckert, R.J.Goldstein. Heat transfer and friction in tubes with repeated-rib roughness. Int. J. Heat Mass Transfer 14, 601-617, 1971.

[3] E.M.Sparrow, W.Q.Tao. Enhanced heat transfer in a flat rectangular duct with streamwise-periodic disturbances at one principal wall. Trans. ASME J. Heat Trans. 105, 851-861, 1983.

[4] Y. T. Chen, J. H. Nie, B. F. Armaly, H. T. Hsieh. Turbulent separated convection flow adjacent to backward-facing step-effects of step height. Int. J. Heat Mass Transfer 49, 3670-3680, 2006.

[5] V.C.Patel, J.Y.Yoon, Application of turbulence models to separated flow over rough surfaces.ASME J. Fluids Eng. 117,234-241, 1995.

[6] D.C.Wilcox, Turbulence Modeling for CFD, second ed. DCW industries, 1998.

[7] T.Hayase, J.A.C. Humphrey, R.Grief.A consistently formulated QUICK scheme for fast and stable convergence using finite-volume iterative calculation procedure. J. Comput. Phys. 98,108-118, 1992.

[8] J.C.Vogel, J.K.Eaton, Combined heat transfer andfluid dynamic measurments downstream of a backward facing step.Trans.ASME J.Heat transfer 107,922-929, 1985.

[9] L.E.Drain, S.Martin.Two component velocity measurement of turbulent flow in a ribbed-wall flow channel In: International Conference on Laser Anemometery-Advances and Applications, Manchester, UK, 1985.

[10] H.C.Chen, V.C.Patel, Near-wall turbulence models for compelex flows including seperation.AIAAJ.26, 641-648, 1988.

[11] J. Nikuradse. Laws for flow in rough pipes. NACA TM, 1292, 1950.

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Promoting Life Style among Sheltered School Children in Banha City, Qualiobia Governorate

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Abstract: Shelter is a place affording protection against danger, or it is a structure that provides privacy and protection for children who have no fixed night time residence. This study aims to evaluate the effect of nursing intervention on promoting life style of school children's shelters in Banha City, Qualiobia Governorate. Design: A quasi experimental design was used to conduct the study. Setting: This study was carried out at two shelters, affiliated to social welfare institutions for boys and girls in Banha City in the academic years 2009 /2010. Sample: All children 55: 36 boys &19 girls in primary, preparatory and secondary school ages (6-18 years). Tool: Three Tools were used for data collection : 1) An interviewing questionnaire for the children concerning socio-demographic characteristics,2) Assessment of health problems: physical, social, psychological and emotional problems: (a)Psychological tests as Fear and Anxiety Test, Poor Relationships, Depression Test, and Emotional Test),(b) Social problems: violence Test, Withdrawal Test and Sexual Abuse Test, 3): Life Style Assessment Sheet. Results: Revealed that the majority of the studied subjects were males, more than two thirds had basic education, according to their health problems, there were statistically significant improvements after the intervention .Concerning self health responsibilities, the mean was 31.222, 32.053 pre program which improved to 50.456, 50.150 after program, the nutritional awareness mean was 28.139, 29.158 pre program, improved to 34.540, 35.150 with a significant difference at p= <0.001. Statistically, there were improvements after the intervention program in physical activity, stress management and environmental safety. Coclusion: The results revealed a significant effect of the intervention program in promoting life-style and providing favorable impact on the health condition of children's shelters. The finding of this study recommended the need for integration between Ministry of Health and Ministry of Society Affairs and Solidarity to develop health care services such as providing periodic check up under supervision of the Ministry of Health and providing shelters with nurses working for 24 hrs/day.

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Keywords: shelter children's, health needs, health problems, life style promotion

1. Introduction

Homeless children are linked to poverty, changes in the housing market, changes in rendering mental health services, persons suffering from mental illnesses, increase of birth rate and personal crisis (**WHO, 2005**).

The number of homeless children around the world has reached more than 150 million. Around 40 million are in Latin America, about 25 to 30 million in Asia, and more than 10 million in Africa. It has been reported that there are 75 million of homeless children in developing countries only, and 5 million in developed countries (**Abd-Elhaleem, 2004)**.

In Egypt, the phenomenon of homeless children is widespread and the absolute number has increased lately. The number of homeless children was estimated to be two million, with 250,000 in Cairo only, and 2172 in Alexandria, homelessness has been officially acknowledged as a social problem, and has been put into consideration by local and international development planners and makers, to be one of their most critical challenges. Accordingly, organizations and associations are working to confront this problem (**Ministry of Society Solidarity, 2006).**

There are numerous factors that contribute to homelessness, common poverty, eroding work opportunities and housing, decline in public assistance, lack of affordable health care, domestic violence, mental illness, addiction disorders, increase incidence of infection, higher infant mortality and morbidity, increased growth of retardation and developmental delay (**Nancy, 2008).**.

Homeless children are more likely to suffer from acute health problems, other than from chronic conditions; the most common illnesses in children are upper respiratory infection, minor skin infection, ear infection, gastrointestinal problems, trauma, eye disorders, and lice infections as might be expected in families that are moving frequently .Homeless children are often at the rear their immunizations, without easy access to health care services (**Judith, 2005).**

Shelters managers should try to improve life style of homeless children, promoting multi service programs in services, and provide health education to all individuals about the importance of adequate housing, good nutrition socioeconomic, effect of drugs, importance of bringing mental health care, and control of birth rate (**Judith, 2005**).The community nurse plays an important role in preventive interventions for children's shelters. Because of the wide range of problems presented by children's shelters, nurses are required to be able to identify factors contributing to or exacerbating homelessness among children and adopt problem solving techniques to face these problems **(WHO, 2004).**

The community nurse plays an important role in such preventive interventions by homeless children , because of the wide range of problems presented by homeless children, nurses are required to be able to identify factors contributing to or exacerbating homelessness among children, and adopt problem solving techniques to face these problems (**WHO, 2004**).

Magnitude of the problem:

The number of homeless children around the world has reached more than 150 million. More than 10 million in Africa. It has been reported that there are 75 million of homeless children in developing countries only. The shelter children number in Arab world is 15 million child and 2, 5 million in Egypt **(Ministry of Social Affairs & Solidarity, 2006).** 50% of the deaths, throughout the world each year, are due to wide range of problems and unhealthy life style presented by homeless children, so, those children need for more information and counseling for health promoting behaviors **(Salem, 2004).**

Aim of the study:

The study aimed to evaluate the effect of nursing intervention on promoting life style of school children's shelters in Banha City Qualiobia Governorate through:

1-Assessing health status among sheltered school children.
2-Assessing healthy life style of sheltered school children regarding promoting healthy and unhealthy behaviors.
3-Identifying health resources and community health services directed to sheltered school children.
4-Developing a nursing intervention program to promote their life style
5-Evaluating the effect of the nursing intervention.

Hypothesis

Nursing intervention will improve life style of sheltered school children.

2. Subjects and Methods

Design:

A quasi experimental design was utilized to conduct the study.

Setting:

The study was conducted in two shelters, affiliated to social welfare institutions for boys and girls, in Banha City Qualiobia Governorate. These shelters were selected because their children's age ranged between 6-18 years old, and they were in different educational levels of primary, preparatory and secondary schools.

Sample:

There are two governmental shelters in Banha City. They accept children aged between 6 to 18 years. The actual number of children in these shelters is 55 (36 males &19 females). All children of both sexes were in primary, preparatory and secondary schools. Their ages ranged between 6-18 years, in these shelters were selected as study sample.

Tools of data collection:

Three Tools were used for collecting data based on literature review and experts' opinion:

Tool (1) An Interviewing schedule to assess: **Socio demographic characteristics of:** shelter's homeless children as age, sex, education and family members including educational level of mother and father, if found and job of father or mother .

Tool (2): Assessment of health problems Homeless children:

Physical problems:

This part include105 close- ended questions (questions 1-80) about physical complaints through medical history as: gastrointestinal, respiratory, renal problems, chronic diseases, communicable diseases, nutritional problems and surgical diseases, as well the same physical problems for acute problems.

Social, psychological and emotional problems: This part is composed of the following tests:

Psychological test as: Fear and Anxiety Test. This part is composed of 27 close- ended questions (questions 1-27) scored according **to Castaneda, et al. (2006**) as: 1-9 free, 10-18 average, 19-27 high rates of fear and anxiety.

Poor relationships with others: this part composed of 12 items, scored according to **Elrakhawy (2000**) as follows: from 1-16 low, 17-32 average, 33-4 high rate of relation.

Depression test:
This part composed of 27 multiple choice of 3 levels, scored according to **Maria (2000**) as follows: from 1-23 free, 24-47 average, 48-71 high rate of depression.
Emotional test:
It contains 4 close and open questions (questions 1-4) scored yes (1) and no (0) marks.

Social problems:
This part composed of more than one test is covering:

Violence test: was composed of 29 statements using variable scale of 3 levels, scored according to psychology today, (**Elrakhawy, 2000)**, from 1-20 free, 21-40 average, 41-58 high level. Theft: this part is composed of 11 questions scored as follows :1-7 free, 8-14 average, 15-22 high level.
Withdrawal test: This part was composed of 23 statements using variable scale of 3 levels, scored according to **Chess. (2000)** from 1-7 free, 8-14 average, 15-23 high rate of grasping.

Sexual abuse test:
This part was composed of 11 statements using variable scale of 3 levels, scored according to Psychology today, (**Chen, etal, 2006)** from 1-7 free, 8-14 average and 15-22 high.

Tools (3):
Life style assessment sheet **(Cookfire, 1991):** to assess children knowledge and practices about healthy life style .It covers five major areas: Self health responsibility including (18) questions, with total scores(36); eating habits and nutrition awareness including (13) question, with total scores(26); environmental safety (12) questions, with total scores(24); physical activity including (7) questions with total scores(14); and stress management including (14) questions, with total score(28). Students responses were scored as follows; always=2, Sometimes=1, and never=0.The researchers categorized student perception as satisfactory when the score is 75% or more, and unsatisfactory when the score is less than 75%.

*Validity test was done through experts from faculty members of Community Health nursing.
4) Operational design:

Pilot study:
The pilot study was carried out on 20 children, who were excluded from the main study sample. They were chosen to test practicability, clarity and simplicity of the tools used, after detection of difficulties that might arise. Some questions were added (e.g, rest & activity), others were clarified (tobacco) or omitted (e.g, general appearance). It took about one month from January to February 2009

Field work:
The process of data collection was carried out in the period from March to April 2009, three days weekly for three hours/daily. Implementation of the educational program from May to September 2009, three days weekly for three hours/daily. An official permission was obtained from Ministry of Social Affairs and Solidarity to the directors of the selected shelters .The aim of the study was explained to them.

Intervention program construction:
It consisted of three phases:
First, preparatory phase and assessment phase:
A review of recent, current, national and international related literature in various aspects of the problem was done at this phase. The aim was to design the study tools and to be acquainted with various aspects of the problem.

Second, planning and implementing phase:
General objective:
The objective of nursing intervention program was to promote life style of children's shelters**.**
The program was designed by the researchers including knowledge related to: personal hygiene, prevention of infection, practicing physical exercise, proper nutrition, sleeping and rest, safe use of medication, environmental sanitation and safety, stress management, control of psychological problems, and smoking cessation**.**
The program was implemented over a period of 4 months; it was carried out in 7 sessions (time allowed 8 hours distributed on 7 sessions : 5 hours for theory and 3 hours for practice. The duration of each session ranged from 30 - 90 minutes.
At the beginning of each session, the investigators started by a summary about what was given through the previous sessions and objectives of the new one, taking into consideration using simple and clear language to suit the level of understanding **.**
Different teaching methods were used including lectures, group discussion, demonstration and re-demonstration, and role-play to implement the program.
The educational media were brochures, colored posters, laptop screen show and real objects.
At the end of each session, the children were informed about the content of the next session and its time.

Third, the evaluation phase:

Evaluation was based on scores of acquired knowledge and practices in pre-test and immediate post-test.

Appropriate statistical methods and tests were used for analysis of the results.

Effects of nursing intervention program for promoting life style of school children's shelter's were identified.

Ethical considerations:

Informed consents were taken from the directors of shelters, after explaining the objectives of the study, they were assured that they will not have any harmful effect on children, and that the information will be confidential and they can withdraw from the study at any time without giving any reason.

5) Administrative Design:

The study was carried out with the co-operation of the different levels of authority in the local office of the Ministry of Social Affairs and Solidarity, and directors of school children's shelters in Benha City where the study was conducted. Written permissions for data collection was obtained from the administrative personnel shelters upon submission of formal letters from the Deans of the Faculty of Nursing, Ain Shams and Helwan Universities to different shelters, requesting their approval for conducting this study at these shelters.

6) Statistical Design:

Data were analyzed using the statistical Package for Social Sciences (SPSS) version 10. Qualitative data was presented as number and percent. Comparison between groups was done by Chi-square test. $P < 0.05$ was considered to be statistically significant of results.

3. Results

Table (1): Illustrates that the majority of the studied subjects were males (36) and only (19) were females, 65.45% had basic education, while 29.09% were secondary education, and only 5.45% could read and write.

Concerning father's education, 96.36% were illiterates while 3.64% could not read and write. Regarding fathers work 78.18% were

Peddlers, while 5.45% had technical work and only 3.64% retried. Regarding mother's work 70.91% were housewives, 50.91% considering with whom the child was living in the past live with mothers and step fathers; 27.27% with fathers and step mothers, and only 16.4% were living with friends before being in the shelters. However all of them 100% were in the shelters for 4 years or more.

Table (2): Shows that the most common health problems, among male and female children pre program applications were upper respiratory disorders such as; recurrent tonsillitis (91.67%, & 78.95% respectively), followed by urinary problems such as; enuresis(38.89 % & 89.47% respectively), while skin diseases such as dermatitis allergy represented (52.78%, & 94.74% respectively) . As regards gastrointestinal problems, the same table shows that 41.67% of the male children complained of diarrhea constipation, while 84.21% of the female children complained of anorexia dyspepsia; 55.56% & 78.95% respectively of headache; (55.56% and 63.16% respectively)for extremities pain; (44.44% and 36.84 % respectively) for back aches (41.67% & 68.42% respectively) of joints pain; and 11.11% and 73.68 for numbers of limbs/ muscle cramp .

In relation to sensory system problems, eye inflammation discharge had the highest percent among male and female children (55.56% & 68.42% respectively) followed by ear discharge (36.11% & 42.11% respectively).

Amoeba was found among 75% and 68.42% of male and female children.

Table (3): Shows that, 69.4 % of male children and 89.47% of the female shad severe fear and anxiety pre program, which improved to be 16.67% and 100.00% post program respectively. As regards depression, 72.2% of male children and all of female children had moderate level of depression, which improved to be 27, 78 % and 42.11% post program respectively. The some table shows also that, 55.6% of male children and 78.95% of the females had moderate emotion disturbance, which improved to be 22.2% and 26.32% post program respectively.

As regards violence, 41.67% of male children and 68.42% of female children had moderate violence; which improved to be 27. 78% and 15.79 % post program respectively.

In relation to sexual abuse, the same table shows that 75% of male children and 68.42% of female children suffered from moderate sexual abuse, improved to be 16.67 % and 26.3% post program respectively. Meanwhile, socio psycho sexual problems among sheltered children showed statistically significant differences pre / post program application.

Figure (1): reveals that there were improvements after implementation of the nursing intervention program in all aspects of sheltered students' life style in relation to self health responsibilities, eating habits, environmental safety, nutritional awareness, physical activity, and stress management.

4. Discussion:

Homelessness has been depicted as an adventure, escaping from oppressive conditions as poverty, domestic violence, child abandonment, school dropout, migration and changing family structures. These are considered the most obvious causes for the existence of homelessness among children (Wahdan, 2005).

The socio- demographic characteristics of the present study sample indicates that the children's age ranged between 6-18 years old. This age group is that of late childhood and adolescence, those aged of 6- < 12 years represented almost one quarter of total sample, while more than half o sample aged 12- 15 years with males slightly more than the of females in the same age, (Table 1).The present study results were congruent with Abo- Elnasser., (2003), who reported that, in his study in Cairo Egypt the age of children inside shelters was 6-18 years. However these funding disagreed with Fahmy (2004), who found that age of children inside shelters was only 6-12 years, and Sedik (2000), who reported that half of the sample aged 11-14 years, and that males were more than the females in children's shelters.

Concerning child's education, findings of this study shows that, the majority of the sheltered children were in schools at different levels of education. More than one third of males were in basic education, while slightly more than half of females were in secondary education, and a minority of the of males were in secondary education, as well the minority of total sample could just read and write (Table1). This study finding was in agreement with Abd-Elhaleem (2004), who reported that two thirds of the sample were in different levels of education, especially primary schools, while 8% could just read and write and less than one third did not go to school. The study result revealed statistically significant difference among children's educational levels.

The present study finding showed the distribution of the studied children according to their parent's education, all the males of mothers and fathers were illiterates and the majority of the females parents can just read and write. (Table 1): Concerning fathers` and mothers occupation, more than three quarters of fathers are peddlers, and minority of them are either technical workers or in pension, and the majority of their mothers were housewives (Table 1). The present study finding was in agreement with Elsamaloty (2000), who reported in his study in Cairo that the percent of illiteracy between families of sheltered children increased due to poverty that pushes children to leave family home and go to street. This may be due to many causes such as illiteracy, poverty, divorce, domestic violence; illegal pregnancy and low social income of parents push them to put their children in shelters. Community can support parent to provide care for their child at home.

This result was in accordance with Abd-Elhaleem (2004), who reported in her study in Cairo that , three quarters of their children original residence was in urban areas, while for one quarter, it was in rural areas and most of them were males. Investigating reasons for not living with both parents, it is apparent from (Table 1) that minority of male children said that the main cause was death of one or both parents, another minority reported that mother or father was in prison, while one tenth of female children said that the main cause was death of one or both parents and few mentioned that divorce was the main reason. This result was in disagreement with Kalil (2000), who reported in his study in Cairo that the majority of sheltered children were living with father and mother before. This is in agreement with the present study result about reason for leaving family and living in shelters.

Considering to children health problems, the present study finding before intervention showed that relatively high percentage of the study sample suffered from gastrointestinal problem's which were significantly higher among female children, as around one third of males suffered from, constipation, diarrhea, nausea, vomiting, and abdominal colic. However more than there quarters of females suffered from nausea / vomiting, the majority of them were suffering from anorexia /dyspepsia, the least percentages of both males and females were suffering from bleeding by mouth, (Table 2). The present study finding was in agreement with Mursy (2004) and Mosa (2005), who reported in their studies in Cairo that a group of health problems that faced homeless children were related to respiratory infections, gastrointestinal and skin disorders.

Regarding urinary problems, relatively high percentages of study sample suffered from various urinary problems. More than one third of males suffered from enuresis and had pain during urination, compared with the majority of females who were suffering from enuresis, polyurea and more than half of them suffered from hematurea. Considering cardiovascular problems, more than on quarter of males versus more than two thirds of females suffered from palpation and none of the males versus almost one fifty of the females suffered from varicose veins. There was a significant difference among children (Table 2). This study finding was in agreement with Mosa (2005), who reported in his study entitled among homelessness Social merge of homeless children, the death rate that cardiovascular complaints were most prevalent among homeless children and sometimes urinary problems as involuntary urination.

The present study showed that the majority of sample suffered from upper respiratory problems representing most of the male sample were suffering from tonsillitis, green nasal discharge and the majority from epitasis .Almost two thirds suffered from sores in mouth, nasal obstruction and rhinitis. Most of the female sample suffered green nasal discharge, sores in mouth and epistaxis and more than three quarters were suffering from tonsillitis, nasal obstructive and rhinitis. More than half of males and females suffered from cough and dyspnea. One third of males suffered from chest pain, more than quarter from bronchitis bronchial asthma, while approximately three quarters of females were suffering from chest pain and chronic bronchitis (Table 2).The present study was in agreement with Mursy (2004), and Mosa (2005) who reported that a group of health problems faced homeless children, these were respiratory infections, gastrointestinal and skin disorders. The study revealed that in relation to upper respiratory problems, there were statistically significantly differences among children. This may be due to absence of medical staff, and absence of nurse's role, especially health teaching, and treatment was neglected, which caused spread of respiratory diseases among children.

Regarding children other physical health complaints, their medical reports, revealed that the majority of sample suffered from central nervous problems, as more than half of males and more than three quarters of the females suffered headache. More than two fifths of males suffered from back aches, joints and extremities' pain , and slightly more than tenth complained muscle cramps, while almost two thirds of females suffered from Bach pain and extremities (Table 2). This study result was in agreement with Mursy (2004), who reported in his study on homelessness problems in Cairo that homeless children suffered from central nervous system. The study result revealed that in relation to the central nervous problems there were statistically significant differences among children under study. Children complaints of central nervous problems were due to standing a lot of time during their work, without considering their ages. Sometimes they sleep on the floor without mattress that had main effect on extremities, back and head especially in female shelters.

Regarding skin diseases the majority of children's shelters complained of different skin diseases, as around half of male children suffered from allergy, and abrasions and fissures and more than two fifths suffered from warts, Tenia ,partial alopecia and scabies while only less than fifth complained from increased thickness, and all of female children suffered from abrasions and fissures and most of them from allergy, warts, Tenia and partial alopecia, and more than half complained from scabies and increased thickness (Table 2). The present study results were in agreement with Mursy (2004), who reported that skin problems were the most common problems among sheltered children; however, they were in disagreement with Kareem (2000), who reported that scabies was the most common skin problem related to poor hygiene. The study showed also that skin problems were statistically significantly different among children. This may be due to poor hygiene and carelessness in shelters on the part of social instructors and medical cares, especially in boy's shelters, adding to the ignorance of children concerning healthy habits.

Considering eye problems more than half of the total sample complained of eye problems. More than half of male children suffered from eye discharge/inflammation and around one third suffered from eye squint, ear discharge and ear ache, and less than fifth of them were wearing glasses and had decrease hearing partial and deafness. While more than two thirds of females suffered from eye inflammation and more than half suffered from eye squint, and ear ache more than two fifths suffered from ear discharge, and the minority of them were wearing glasses and had decrease hearing (Table 2). The present study was in accordance with Abd-Elhaleem (2004) and Mosa (2005)), who reported that the most common problems prevailing among homeless children were in sensory system as eye, and auditory complaints. The study shows that skin problems were statistically significantly different among children. This is due to the absence of medical care from doctors and nurses in shelters during child's illness. They also did not follow social supervision. This was specially happened in Elbaneen shelter that lead children to become deaf and miss one eye or two, as a result of ignorance of their state and the required treatment especially when children were young. This led to spread of disease faster among children.

The present study finding showed that parasitic diseases revealed highly statistically significantly difference among children as three quarters of male children suffered from Amoeba, more than half from worm ascaris and few suffered from bilharziasis , while for females more then two thirds of them suffered from Amoeba and worm ascaris and the minority of them suffered from bilharziasis, (Table 2). These results were supported by Mosa (2005), who reported in his study in Cairo that parasitic disease was most common especially bilharziasis disease as a result of bathing in contaminated canals and rivers. This study result was also in agreement with Mursy (2004) who reported that parasitic

diseases were most common among homeless children. This is due to the absence of the nurse health teaching role about hygienic care and healthy habits in children's shelters and schools and medical treatment among children.

In relation to nutritional problems, the present study showed that there was highly statistically significantly difference among children. Relatively high percentages of study sample complained of anemia, the majority of males and more than half of females suffered from thinness and minorities of both males and females suffered from obesity, (Table 2). This study was in agreement with Abd-Elhaleem (2004), who reported that the most common nutritional problem in shelters was anemia as a result of not eating all types of food, leading to thinness. This is due to the children nutritional condition. Shelters where they are offered quantities and qualities less than body requirements and are not supervised under special nutritionist which helps to provide balanced diet to children, in addition to the nutritional bad habits prevailing among homeless children.

The present study showed that in relation to social problems among homeless children in Qualiobia Governorate shelters, highly statistically significant differences were found between male and females children as the majority of males suffered from either mild or moderate violence, with equally percentages and more than two thirds of females had moderate degree of it. In addition more than half of males complained of moderate emotion disturbance, and more than three quarters of females had moderate degree of it. All of males were suffering from moderate withdrawal; as well most of females had high degree of it. While three quarters of males had moderate sexual disturbance, more than two thirds of females had moderate degree, as well (Table 3).This study results were was in agreement with Mursy (2004) and Mosa (2005), who found in their studies in Cairo, Egypt that violence, theft, withdrawal and hyper sexuality are mostly prevalent among children in shelters. Prevalence of social problems among homeless children was due to community disapproval and refusal of them, adding to loss of love from parents and people. Some of them had sense of shame due to their illegality, and without separation between ages inside shelters, which lead to appearance of homosexuality or heterosexuality among children

The present study showed that psychological problems among homeless children in Qualiobia shelters were significantly higher among them. The majority of males suffered from moderate depression, and all of the females had as well moderate of it. All of the females had different degrees of depression, and more than one quarter of males had severe depression leading to poor relations with others, while more than two thirds of males and the majority of the of females suffered from sever fear and anxiety, (Table3). This study was in agreement with Abd-Elhaleem (2004) and Ataka (2005), who reported that depression, poor relations and anxiety were the most, prevailing among homeless children. Which were due to community's refusal of them, in addition to their fear of people's look at them. Some of them had sense of shame due to their illegality.

The present study revealed that there were unhealthy life style among male & female children sheltered pre nursing intervention which improved post nursing intervention in both groups .As regards self health responsibility, the children sheltered expressed unsatisfied self health responsibility pre nursing intervention .This could be due to that child's healthy habits are usually established by parents at home in the early years. It needed reinforcement and repetition throughout their life, that's why they missed it in shelters due to insufficient role of shelter's managers and their team while post intervention it improved (Figure 1). Investigating eating habits/ nutritional awareness, it improved among both males and females after program intervention. (Figure 1). In a study carried out by Morgan (2005) on the role of breakfast in nutrient intake, he reported that 39% of children didn't eat breakfast meal especially if both parents work. In Margan study, this could be due to life style of children before being in the shelters, where the step mother or step father who don't care, or they neglect or don't recognize the importance of taking breakfast for children similarly ,in accordance with the previous studies Bartkien (2007) proper nutrition is essential for health and well-being of children. A properly nourished child is less susceptible to acute illness and is better able to develop physical, intellectual, emotional and social competencies .In relation to physical activity, the study revealed also unsatisfied responses of sheltered children pre nursing intervention, there were significant mean improvements in both male and female children, this could be attributed to nursing intervention in developing and encouraging attitude toward physical exercise teaching the benefits of exercises. This finding was in accordance with Gailahue (2007), who emphasized on the development and maintenance of exercise throughout life.

Considering environmental safety, the means of males and females pre program were unsatisfactory. However, after program implementation, they improved with statistically highly significant differences (Figure 1). In accordance, Mosa (2005) who found that many of the shelters were crowded and were not suitable for shelter safety environment.

As for stress management , sheltered children scores for both males and females were unsatisfactory, while after nursing intervention, they improved considerably with statistically highly significant differences (Figure1). In agreement with the previous finding, Abd-Elhaleem (2004) and Mosa (2005), who reported that stress management, poor relations and anxiety were most, prevailing among sheltered children. was due to community's refusal of them, in addition to their fear of people's look at them. Some of them had sense of shame due to their illegality.

To summarize, there were unhealthy life style among sheltered children as regards healthy life style: Self health responsibility, Eating habits/ nutritional awareness, Environmental safety, Physical activity, and Stress management.

Conclusion

According to the findings and research hypothesis, the current study concluded that; majority of the studied subjects were males (36) and only 19 were females. In relation to their educational level, almost two thirds of the studied subjects had basic education, while more than one quarter of them were secondary education, and a minority only could read and write. Spreading of diseases was prevalent among children's homeless shelters with highly statistically difference between children's pre/post program intervention as regards , many diseases such as in sensory system: sensitive eye ,ear, ache/discharge; in respiratory tract infections (recurrent tonsillitis); in gastrointestinal (abdominal colic's) ;urinary tract infections (burning /pain during urination &enuresis); in central nervous diseases (back aches); and in nutritional diseases (anemia),in addition to environmental problems, where all children suffered from many diseases and bad hygiene. In addition; the building was not suitable, to meet the needs of children.

The nursing intervention program had positive effect on life style of school children shelters, where there were improvements after implementation of the intervention program in all aspects of their life style

Table (1): Socio- demographic characteristics of sheltered children & their parents. (n=55)

Variable	Male (n=36)		Female (n=19)		Total	
	No	%	N0	%	N0	%
Age						
6-	9	25.0	5	26.4	14	25. 4
12-	20	55. 6	10	52.6	30	54.6
15-18	7	19.4	4	21.o	11	20.0
Children education:						
Read and write	3	8.3	0	0.00	3	5. 45
Basic education	27	35	9	47.4	36	65. 45
Secondary education	6	16. 7	10	52.6	16	29.10
Father education:						
Illiterate.	36	100	17	89.5	53	96.36
Read and write.	0	0.00	2	10.5	2	3.64
Father occupation:						
Technical workers	3	8.3	0	0.00	3	5. 5
Peddlers	28	77. 8	15	79.0	43	78.2
Pension	2	5. 6	-	-	2	3.6
Dead	3	8.3	4	21.1	7	12.7
Mothers education :						
Illiterate.	36	100	17	89.5	53	96.36
Read and write	0	0.00	2	10.5	2	3.64
Mother occupation:						
Workers	2	5. 6	12	63.2	14	25. 5
Housewives	32	88. 9	7	36.8	39	70.9
Dead	2	5. 6	0	0.00	2	3.6
With whom you were living in the past:						
Live with mother	2	5. 6	1	5.3	3	5. 45
Live with mother and step father	16	44.4	12	63.2	28	50.91
Live with fathers and step mother	11	30. 6	4	21.1	15	27.24
Live with friends	7	19.5	2	10.5	9	16.40
Duration of living in shelters(in years) :						
1- 4	0	0.00	0	0.00	0	0.00
+ 4	36	100	19	100	55	100

Table (2): Distribution of sheltered children according to their physical health problems pre/ post program application (n=55).

	Male (n = 36)				Female (n =19)					
	Pre		Post		Pre		Post		Chi-square	
Physical Health Problems	No	%	No	%	No	%	No	%	P1	P2
Gastrointestinal :										
Abdominal colic	12	33.33	3	8.33	12	63.16	7	36.84	0.009	0.097
Nausea /vomiting	10	27.78	1	2.78	15	78.95	1	5.26	0.003	0.000
Constipation / diarrhea	15	41.67	5	13.89	13	68.42	1	5.26	0.008	0.000
Anorexia /dyspepsia	10	27.78	4	11.11	16	84.21	3	15.79	0.067	0.000
Bleeding by mouth	4	11.11	1	2.78	6	31.58	1	5.26	0.179	0.045
Urinary problems :										
Burning / difficulty / pain during urination/ dysurea	8	22.22	2	5.56	11	57.89	8	42.11	0.042	0.259
Enuresis	14	38.89	8	22.22	17	89.47	2	10.53	0.100	0.000
Polyurea	11	30.56	6	16.67	16	84.21	3	15.79	0.133	0.000
Hematurea	7	19.44	2	5.56	11	57.89	8	42.11	0.076	0.259
Cardio vascular disorders :										
Palpitation	10	27.78	2	5.56	13	68.42	6	31.58	0.012	0.025
Varicose veins	0	0	0	0	4	21.05	2	10.53		0.330
Upper respiratory disorders nasal cavity and throat :										
Nasal obstruction	24	66.67	12	33.33	15	78.95	4	21.05	0.005	0.000
Rhinitis	24	66.67	12	33.33	14	73.68	5	26.32	0.005	0.004
Black/ green nasal discharge	33	91.67	3	8.33	18	94.74	1	5.26	0.000	0.000
Epistaxis	29	80.56	7	19.44	16	84.21	3	15.79	0.000	0.000
Sores in mouth/bad odor	25	69.44	11	30.56	18	94.74	1	5.26	0.001	0.000
Recurrent tonsillitis	33	91.67	3	8.33	15	78.95	4	21.05	0.000	0.000
Lower respiratory disorders :										
Cough/ dry or productive	19	52.78	10	27.78	11	57.89	8	42.11	0.027	0.259
Dyspnea	18	50	9	25	11	57.89	8	42.11	0.025	0.259
Chest pain/wheeziness	12	33.33	3	8.33	14	73.68	5	26.32	0.009	0.004
Chronic bronchitis /bronchial asthma	10	27.78	4	11.11	15	78.95	2	10.53	0.067	0.000
Central nervous system :										
Back aches	16	44.44	5	13.89	12	63.16	7	36.84	0.004	0.097
Joints pain	15	41.67	5	13.89	13	68.42	6	31.58	0.008	0.025
Extremities pain	20	55.56	6	16.67	12	63.16	7	36.84	0.001	0.097
Numbness of limbs/ muscle cramp	4	11.11	2	5.56	14	73.68	5	26.32	0.337	0.004
Headache	20	55.56	2	5.56	15	78.95	4	21.05		0.000
Skin diseases :										
Dermatitis / allergy	19	52.78	5	13.89	18	94.74	1	5.26	0.000	0.000
Warts	16	44.44	1	2.78	17	89.47	2	10.53	0.000	0.000
Abrasions& fissures /wound	18	50	5	13.89	19	100	4	21.05	0.001	0.000
Tenia / Tenia capitals	16	44.44	5	13.89	16	84.21	3	15.79	0.004	0.000
Partial alopecia	17	47.22	5	13.89	16	84.21	3	15.79	0.002	0.000
Scabies	16	44.44	6	16.67	10	52.63	1	5.26	0.010	0.002
Increased thickness/ roughness	7	19.44	2	5.56	11	57.89	3	15.79	0.076	0.009
Sensory system :										
Eye squint /blurred vision	12	33.33	3	8.33	10	52.63	3	15.79	0.009	0.019
Eye inflammation / discharge	20	55.56	3	8.33	13	68.42	3	15.79	0.000	0.001
Wearing glasses	7	19.44	0	0	3	15.79	0	0	0.006	0.115
Auditory complaints										
Decrease hearing (deaf / partial	4	11.11	0	0	3	15.79	0	0	0.057	0.115
Ear discharge / wax, blood	13	36.11	3	8.33	8	42.11	1	5.26	0.005	0.009
Ear ache	12	33.33	5	13.89	11	57.89	8	42.11	0.047	0.259
Parasites :										
Bilharzias	5	13.89	0	0	4	21.05	0	0	0.027	0.053
Amoeba	27	75.00	9	25	13	68.42	6	31.58	0.000	0.025
Worm ascaris	20	55.56	16	44.44	13	68.42	6	31.58	0.240	0.025
Nutritional diseases:										
Anemia	22	61.11	12	33.33	10	52.63	4	21.05	0.016	0.046
Thinness	30	83.33	5	13.89	10	52.63	8	42.11	0.000	0.373
Obesity	6	16.67	0	0	3	15.79	0	0	0.012	0.115

Table (3) Distribution of sheltered children according to their social, psychological and sexual problems pre /post program application(n= 55).

Socio-psycho sexual Problems	Male (n = 36)				Female (n =19)				Chi-square	
	pre		post		pre		Post			
	No	%	No	%	No	%	N0	%	P1	P2
Fear and anxiety :										
Mild	0	0.00	20	55.56	0	0.00	18	94.74	0.096	0.011
Moderate	11	30.56	10	27.78	2	10.53	1	5.26		
Severe	25	69.44	6	16.67	17	89.47	0	0.00		
X^2(P-value)	31.693(0.000)				35.333(0.000)					
Depression:										
Mild	0	0.00	21	58.33	0	0.00	11	57.89	0.011	0.181
Moderate	26	72.22	10	27.78	19	100.00	8	42.11		
Severe	10	27.78	5	13.89	0	0.00	0	0.00		
X^2(P-value)	29.778(0.000)				15.481(0.000)					
Emotion disturbance :										
Mild	0	0.00	23	63.89	0	0.00	12	63.16	0.086	0.905
Moderate	20	55.56	8	22.22	15	78.95	5	26.32		
Severe	16	44.44	5	13.89	4	21.05	2	10.53		
X^2(P-value)	33.905(0.000)				17.667(0.000)					
Violence:										
Mild	15	41.67	25	69.44	3	15.79	15	78.95	0.117	0.571
Moderate	15	41.67	10	27.78	13	68.42	3	15.79		
Severe	6	16.67	1	2.78	3	15.79	1	5.26		
X^2(P-value)	7.071(0.000)				15.250(0.000)					
Withdrawal :										
Mild	0	0.00	18	50.00	1	5.26	14	73.68	0.165	0.090
Moderate	36	100.00	18	50.00	18	94.74	5	26.32		
Severe	0	0.00	0	0.00	0	0.00	0	0.00		
X^2(P-value)	24.000(0.000)				18.614(0.000)					
Sexual abuse :										
Mild	9	25.00	30	83.33	6	31.58	14	73.68	0.602	0.395
Moderate	27	75.00	6	16.67	13	68.42	5	26.32		
Severe	0	0.00	0	0.00	0	0.00	0	0.00		
X^2(P-value)	24.671(0.000)				6.756(0.009)					

Table (4): Distribution of sheltered children according to their healthy life style pre / post program application(n=55).

Healthy Life style	Male (n=36)						Female (n=19)						T-test	
	Pre			Post			Pre			Post				
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	P1	P2
Self health responsibility	31.222	±	3.490	50.456	±	1.450	32.053	±	2.549	50.150	±	1.120	0.364	0.427
Paired t-test(P)	<0.001*						<0.001*							
Eating habits /nutritional awareness	28.139	±	5.083	34.540	±	2.112	29.158	±	1.675	35.150	±	1.751	0.401	0.286
Paired t- Test (p)	<0.001*						<0.001*							
Environmental safety.	28.646	±	1.211	33.150	±	2.354	26.213	±	2.330	35.123	±	2.025	0.000	0.003
Paired t-test(P)	<0.001*						<0.001*							
Physical activity	22.560	±	2.310	32.213	±	3.213	25.330	±	2.321	34.000	±	1.231	0.000	0.024
Paired t-test(P)	<0.001*						<0.001*							
Stress management	22.130	±	2.354	38.654	±	2.133	26.133	±	3.513	36.554	±	4.250	0.000	0.018
Paired t-test(P)	<0.001*						<0.001*							

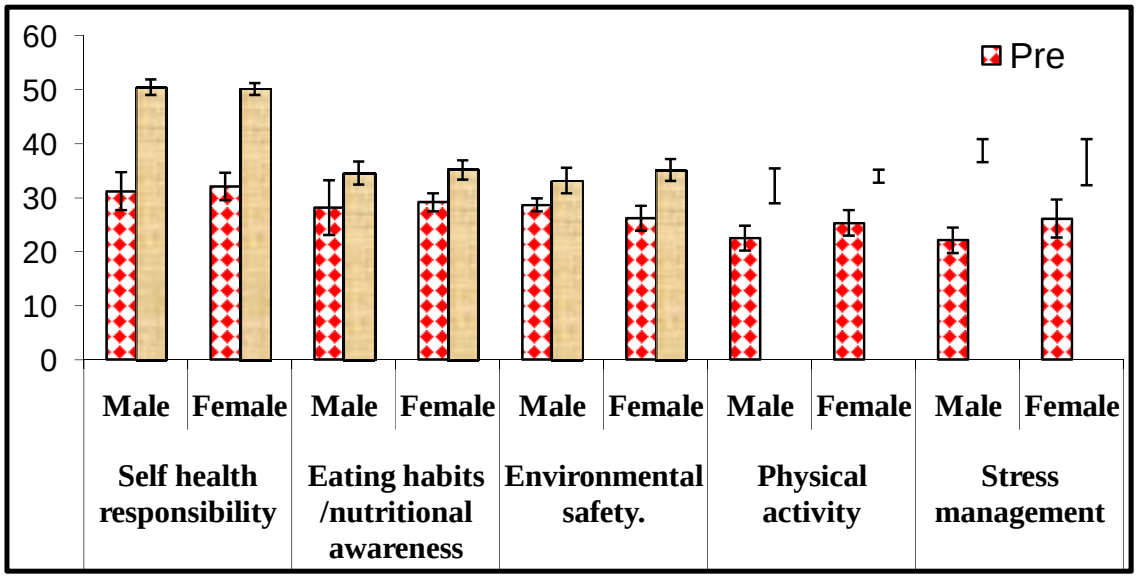

Figure 1. Considering environmental safety, the means of males and females pre-program were unsatisfactory. However, after program implementation, they improved with statistically highly significant differences.

Recommendation

Based on the findings of this study, the following suggestions can be recommended for health and policy considerations:

- Cooperation between the Ministry of Health and Ministry of Society Solidarity to develop health care services such as; providing periodic check up under supervision of the Ministry of health and providing nurses inside shelters for 24hrs/day not as health visitors.
- The Ministry of Social Affairs and Solidarity has to separate between children aged 6 -<15 years in shelters and 15-18 years in other shelters. The necessary of separation is to facilitate their supervision and provide them with suitable care according to health needs in those two age group categories
- Providing health education for sheltered children about safety measures to protect them from health hazards and injuries.
- Increasing people's awareness about sheltered children and their needs, and how to control them inside community through conferences, meeting, mass media and programs.
- More researches are needed to identify common health problems among children in shelters and resources that meet needs of those children

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References

1. Ataka,M, (2005):The issues of population, family and childhood, street children (social problems). High Institute of Social Services, Alexandria, pp. 20-33.
2. Abo-Elnasser, M.(2003):The role of voluntary organizations in coping with the street children problems, in Egypt. The Fifth Annual Conference for the Faculty of Nursing. Ain Shams University, Cairo 8-10 Nov 2003.
3. Abd-Elhaleem, N. (2004): Social repercussion of the phenomena of street children, homelessness, health examination on needs and resources. The National Planning Institute, pp20-45.
4. Bartkien, M. (2007): standard weight table and food composition.2005, available at www. WHO.com.
5. Chess, S.(2000):An introduction to child psychiatry.4th ed, New York, Grune&Stratton.Pp.39
6. Chen, X. Hoyt, D. Tyler, K .and Beek, L. (2006): Early sexual abuse, street adversity, and drug use among homeless children and runaway adolescents in the Midwest. Journal of Drug Issues 22,(4):Pp.1-22.
7. Cookfair, J.M. (1991): Nursing Process and Practice in the Community, Mosby, St.Lpuis; Pp. 80-83.
8. Castaned M. and Balrmo A.(2006): Fear and anxiety measurement, Elnahda library,

adolescence health journal.Pp.53-62. Available at www.psychtoday.com.9/3/2008
9. Elrakhawy, Y. (2000): Anxiety, depression and relationship measurement, available at www.psychtoday.Com.
10. Elsamaloty, A. (2000): Deviant behavior of children homeless; Study of the culture of stranded house Knowledge. Cairo University
11. Fahmy, S. (2004) :The tragedy of civilization in the third millennium homelessness problems, theft of savings among homeless children the Mode University office,Alexandria,1st ed. Egypt. Available at www.middle-east-online.com//id=27813 format 2008.19/1/2008
12. Gailahue, M. (2007): Social and Physical Health of Homeless children Previously Treated for Mental Health Problems. Hospital and Community Psychiatry 39:Pp . (510-516).
13. Judith, C. (2005): Community health nursing, role of community health nurse among homeless children, (16th ed). pp. 122-144. File: IIF:/ Disease Risk Analysis- Africa. Htm Accessed on 1/1/2008
14. Kalil, A. (2000): Homelessness in Arabic World, Cairo, Arabic Commit for Childhood and Development.
15. Kareem, A. (2000): Children in difficult circumstances-working children and street children, Cairo, National Council for Childhood and Motherhood.
16. Maria, K. (2000): Depression measurement , Ph D. Western Psychiatric Institute and Clinic, University of Pittsburgh, U.S.A
17. Morgan, R.(2005) : The Effect of poverty on children. Future Child 7:Pp. (55-71).
18. Ministry of Social Affairs & Solidarity in Dakahlia (2006): Psychological needs. Accessed at http://www.Homelessness needs/en. Wikipedia.org/wiki/streetchildrenangels philippiness/2007/yahoo.Com. Accessed on 5/6/2007
19. Ministry of Social Affairs &Solidarity, (2006): Homeless shelter in Egypt, Available at: http;/ includes. Nurse. Com/ apps/ pbcs. Dll/ article? Sources and services offered to homeless shelters//AID Accessed on=(2006).
20. Mosa, M. (2005): Social merge of homelessness children , the death rate among homelessness. PhD Thesis Faculty of Social Services, Mansura University, (1st ed), Egypt, pp. 17-40.
21. Mursy, A. (2004): Homelessness problems, vision of an uncivilized, the Egyptian Elnahda Library, Cairo, (1st ed), pp.146
22. Nancy, K. (2008): Factors contributing to homelessness, a glossary for social epidemiology. PhD Harvard University School of Public Health & Journal of Poverty Accessed at www camillushours, org,on. 1/2/2008.
23. Salem, M. (2004): The problems of street children. Research published in the Scientific XIII Conference of the Faculty of Social Services, Helwan University.
24. Sedik, A. (2000): Experiences in homeless children in Egypt, Cairo Center to Protect Rights of Children.
25. Wahdan, A. (2005): Homelessness, National Center for Social and Criminal Research. Economic Factors, Cairo.
26. World Health Organization (WHO) (2005): Homeless children needs. Available at www.unicef. Org/crc/2007.
27. World health Organization (WHO) (2004): Child labor, health effects of combined exposure in the work environment, Report of WHO expert committee. Geneva: Technical report no; 9-56 Available at: http//; www. Child labor/ definition/health effects of combined exposure in the work environment.

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Perinatal Eexposure to Cadmium Affects Neurobehavioural Development and Anxiety – Like Behaviour in Rat Offspring

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Abstract: Cadmium is a known industrial and environmental pollutant. The present study was conducted to assess the potential influence of maternal cadmium (Cd) exposure on postnatal development and neuromotor maturation in offspring rats. Moreover, locomotors activity and anxiety – like behavior was also monitored post weaning. Cadmium chloride in doses of 0 , 5, 50 mg / L was administered orally in drinking water to pregnant rats from the 7th day of pregnancy till weaning of these pups at 30 days of age . All the females were allowed to deliver and wean their offspring. The pups were evaluated for physical development and neuromotor maturation (Reflexes). Also, open- field activity and anxiety- like behavior in elevated plus maze (EPM) were determined at weaning age of young rats. The results revealed that, birth weight of pups exposed to high doses of Cd was decreased relative to controls. A delay in some developmental landmarks (incisor eruption, vagina opening, testes descent) due to maternal cadmium exposure was also noticed pups. Moreover, a delay in neuromotor development (neonatal reflexes) and poor motor coordination was recorded in $Cdcl_2$ exposed neonates. Cadmium – exposed offspring showed hyperactivity in open field test presented by increased horizontal locomotion. Anxiogenic effect of cadmium was evidently observed during open –field and elevated plus maze tests. Our results strongly suggest that maternal exposure to $Cdcl_2$ in high doses has detrimental effects on the physical maturation & reflexes of neonate rats as well as anxiety – like behavior.

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Introduction

Cadmium (Cd) is a widespread toxic environmental and industrial pollutant. It is listed by the U.S. Environmental Protection Agency as one of 126 priority pollutants. In human, the primary route of exposure is via contaminated drinking water, food supplies, or tobacco. Normally, the main source of exposure to toxic compounds during the neonatal and first part of the infancy period is breast milk. This period is characterized by rapid growth and development of the nervous, immune, and endocrine / reproductive systems which render the newborn more vulnerable than adults to harmful substances (Rice & Barone Jr, 2000). The embriotoxic action of cadmium has been observed both in human studies and in animal experiments. In animals, cadmium can be transported across the placenta and excreted via the milk. Consequently, fetuses and pups can be exposed to Cd during gestation and lactation (Antonio et al; 1998).As the central nervous system of newborn animals is very susceptible to cadmium, the developmental pattern of certain neurochemicals in developing rat after Cd exposure during gestation and lactation periods was studied to explore the possible mechanism of Cd – induced neurotoxicity in growing animals (Desi et al; 1998). Lack of systematic studies on the effects of pre- and postnatal cadmium exposure on behavioural and functional outcomes prompted us to clarify the effects of cadmium chloride exposure during pregnancy and lactation on behavioural development & anxiety – like behavior. This will help, to find sensitive biomarkers that can be used to signal the early risk of cadmium intoxication.

2. Materials and methods:

2.1. Animals and housing:

Thirty six pregnant female Wistar rats were obtained from the Unit for Laboratory Animals at Faculty of Veterinary Medicine, Cairo University. They were maintained in plastic cages, with stainless steel wire lids (bedded with wood shavings), on a standard laboratory feed diet. Feed and water were offered ad libitum. Rats were housed at a controlled temperature of 21 ± 1ºC, 60 % humidity and under a 12-h-light: 12-h-dark schedule. All efforts were made to minimize the numbers of animals and their suffering in this study through following the guidelines released by Cairo University Policy on Animal Care and Use.

2.2. Administration of cadmium chloride:

Pregnant females were divided at random into three groups of 12 each and received $cdcl_2$ in crystalline form (Sigma Aldrich) at one of three

different concentrations; 0 (control), 5 mg /L (low dose) and 50 mg / L (high dose) (Waalkes *et al*.,1999). Cadmium chloride was incorporated in drinking distilled water and administered to pregnant rats ad libitum from day 8 of gestation till termination of lactation and weaning of pups. The pups were exposed to cadmium during the suckling period via milk and post weaning via drinking water.

2.3. Physical testing of offspring:

Pregnant rats were allowed to deliver and wean their offspring. At birth all pups were weighed and again at the age of weaning (on day 30).The Pups were evaluated for physical maturation, the day of occurrence for pinna detachment, incisor eruption, eye opening, fur growth. Also, vagina opening and testes descent were monitored.

2.4. Sensorimotor reflexes (neuromotor maturation):

The functional and behavioural developmental parameters were measured and scored for all individual offspring during the lactation period (Tanaka, 2006) and were analyzed on score frequencies. The measured variables were as follows:

* Righting reflex test: was conducted on postnatal days 4,5 and 6. Pups were placed on their back on a horizontal board and were released .Time to return to the normal dorso-ventral position was measured.

* Negative geotaxis test: was conducted on postnatal days 7, 8 and 9 in which the animals were placed on 30° inclined screen, and the time spent turning upward was recorded.

**Cliff avoidance: was assessed on postnatal days 7,8 and 9, by placing the animal on the edge of a bench, with nose and forepaws just over the edge. Time to move away from the edge of a bench was recorded.

*Startle responses: when a loud clap of the hands occurs less than 10 cm away, the pup shows a whole body startles response.

* Vibrissa reflexes: when a cotton swab is stroked across the rat's vibrissa (whiskers), it places its paw on the cotton swab.

Both startle response and vibrissa reflexes are scored as 0 (no response), 1 (a slight response), and 2 (a complete response) (Meer and Raber, 2005).

*Balance test: on day 19, the ability to balance and move along the rim of a 2-liter beaker was tested, as described by Smart and Dobbing (1971). Percent of animals that fell from the rim was calculated and compared between groups.

2.5. Post- weaning behavioural tests:

At 30 days of pup's age, behavioural tests were performed in the first half of light phase of the light/dark cycle. All behaviours were scored by a single trained observer unfamiliar with treated animals. Hand operated counters and stop watches were used to score animals' behaviour.

2.5.1. Open field behavior test:

The open field test provides simultaneous measures of locomotion, and anxiety (Kelly, 1993; Millan, 2003). The open field used was a square wooden arena measured (90 x 90 x 25cm).The wood of the apparatus is covered with a plastic laminate (formica), which prevents absorption of fluids (urine of rats). The floor was divided by black lines into 36 small squares (15 x 15cm). The open field maze was cleaned between each rat using 70% ethyl alcohol to avoid odor cues. The rats were carried to the test room in their home cages and tested once at a time for 5 minutes each. Rats were handled by the base of their tails at all times. Rats were taken from their home cages and placed randomly into one of the four corners of the open field facing the centre. The behavioural scores measured in this experiment included total numbers of line crossings (number of squares), rearing against the wall, and fecal boli as well as the time spent freezing (no movement) was quantified.

2.5.2. Elevated plus maze test:

The elevated plus-maze was used for testing of anxiety and emotionality. The degree of avoidance of the open arms of the maze has been considered as a measure of strength of fear drive (Trullas and Skolnick, 1993). The apparatus consists of 4 crossed arms, two open arms (50 x 10 x 30 cm) and two closed arms (50 x 10 x 30 cm). The maze was elevated 65 cm above the floor. The rat was placed in the centre of the maze and the number of entries in open and closed arms, respectively, as well as the time the animal spent in the open and enclosed arms during a period of 5 min test session was recorded (Kierstin, 2003; Walf and Frye, 2007). After each trial the maze was wiped out with a cloth dipped in 70% ethyl alcohol and allowed to dry.

2.6. Statistical analysis

Statistical analyses were performed by using SPSS statistical software package (SPSS, 2006). Data are presented as means with their standard error. Normality and homogeneity of the data were confirmed before ANOVA, differences among the experimental groups were assessed by one-way ANOVA followed by Duncan's test.

3. Results:

3.1. Physical development:

Physical maturation of pups all over the study was shown in Table 1. Lower body weight at birth

was significantly seen in groups of pups exposed to high concentrations of $Cdcl_2$ compared to those exposed to low doses and pups in control group. Where no differences were observed in body weight at weaning age between control and experimental pups. Developmental landmarks were evaluated for all pups by a two-way ANOVA. No significant differences on pinna detachment or on the number of days at eye opening or fur growth . Where , there was a differences between groups on the number of days at incisor eruption . Moreover, effect of Cd was observed in both males and females in sexual maturation (vagina opening in females and testes descent in males) with significant differences between groups.

3.2. Neuromotor and neurobehavioural maturation

The effect of cadmium on neurobehavioural development in rat pups was demonstrated in Table 2,3,4,5,6. The righting reflex differed between groups, where a delay in this physical characteristic occurred in the Cd treated group ($P< 0.05$). The negative geotaxis reflex showed delay in the high Cd group ($P< 0.05$) when compared to the control group . A significant delay ($P< 0.05$) in cliff avoidance reflex was observed also between groups exposed to cadmium, especially high doses, and the control group. Moreover, pups exposed to $Cdcl_2$ showed delay in startle response as well as vibrissa reflex. Concerning Balance and coordination, a significant impairment in motor balance was noticed in rats treated with cadmium (increased percent of animals that fell from the rim of beaker) (Table, 7).

3.3. Open field test:

The effect of cadmium treatment on parameters of open field test was illustrated in Table, 8. Rats under cadmium intoxication increased significantly the mean covered distance in the open field test when compared with the control group. An anxiogenic like effect was obtained in cadmium-exposed rats when compared to their counterparts in controls. Cadmium -treated individuals presented a significant increase of rearing in peripheral area of the test. Also, administration of $Cdcl_2$ to rats produced an anxiogenic profile of behavioural changes as indicated by increased time spent freezing. Moreover, a marked significant increase in fecal boli was also observed in rats following cadmium treatment when compared to animals belonging to control group.

3.4. Elevated plus maze test (EPM):

The effect of cadmium on measurement of elevated plus maze was demonstrated in Table, 9. Animals under cadmium effects significantly diminished the numbers of entries in the open arms of the maze, accompanied with significant increase of this measure in the closed arms. Regarding time spent in the open arms, cadmium was significantly successful in endorsing an aversive dose-related effect since the shortest time spent in open arm was recorded with high cadmium group.

Table (1) : Effect of $Cdcl_2$ on physical maturation of the offspring of prenatally exposed rats.

Parameters / groups	Control	Low	High
-Birth weight (gm).	6.57 ± 0.37^{ab}	6.17 ± 0.46^{ab}	3.72 ± 0.46^{c}
-Weight at weaning (gm).	28.37 ± 1.30^{abc}	29.56 ± 1.76^{abc}	31.90 ± 2.1^{abc}
-No. of days at pinna detachment.	2.8 ± 0.40^{abc}	2.75 ± 0.43^{abc}	3.00 ± 0.00^{abc}
-No. of days at incisor eruption.	3.00 ± 0.00^{ab}	3.75 ± 0.43^{ab}	2.43 ± 0.50^{c}
-No. of days at fur growth.	7.2 ± 0.40^{abc}	6.88 ± 0.60^{abc}	7.00 ± 0.58^{abc}
-No. of days at eye opening.	14.6 ± 0.40^{abc}	14.8 ± 0.50^{abc}	15.8 ± 0.64^{abc}
-No. of days at testes descent.	22.9 ± 0.60^{ab}	23.8 ± 1.10^{ab}	26.8 ± 2.50^{c}
-No. of days at vagina opening.	30.9 ± 4.10^{ab}	32.5 ± 3.60^{ab}	42.9 ± 5.40^{c}

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA .
Values represent mean ± SEM.

Table (2): Effect of $Cdcl_2$ on the righting reflex of neonates .

Day/groups	Control	Low	High
4day	3.83 ± 0.76^{a}	2.41 ± 0.21^{bc}	2.45 ± 0.25^{bc}
5day	1.35 ± 0.09^{ab}	1.60 ± 0.15^{ab}	2.34 ± 0.37^{c}
6day	1.38 ± 0.14^{ab}	1.25 ± 0.07^{ab}	2.39 ± 0.06^{c}

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA .
Values represent mean ± SEM.

Table (3) : Effect of $Cdcl_2$ on the negative geotaxis of neonates.

Day/groups	Control	Low	High
7day	9.03±0.90^{ab}	10.73±0.88^{ab}	15.18±1.57^{c}
8day	10.03±1.46^{ab}	9.79±0.76^{ab}	13.86±2.04^{c}
9day	10.07±2.03 ^{ab}	9.47±98 ^{ab}	13.70±1.01^{c}

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA .
Values represent mean ± SEM.

Table (4) : Effect of $Cdcl_2$ on the cliff avoidance of neonates .

Day/groups	Control	Low	High
7day	4.76±0.60^{ab}	4.64±0.34^{ab}	6.94±0.65^{c}
8day	2.74±0.38^{ab}	2.95±0.23^{ab}	6.14±0.86^{c}
9day	2.71±0.41^{ab}	3.77±0.43^{ab}	6.71±1.47^{c}

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA .
Values represent mean ± SEM.

Table (5) : Effect of $Cdcl_2$ on the startle response of neonates (% of neonates).

Scores	Control(n=28)	Low (n=49)	High (n= 28)
0 (no response)	00.00(0)	2.04(1)	00.00(0)
1 (slight response)	17.85 ^{a} (5)	51.02 ^{bc} (25)	60.71 ^{bc} (17)
2 (complete response)	82.14 ^{a} (23)	46.94 ^{bc} (23)	39.29 ^{bc} (11)

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA .
Values represent mean ± SEM.

Table (6) : Effect of $Cdcl_2$ on the vibrissa reflexes of neonates (% of neonates).

Scores	Control(n=26)	Low (n=49)	High (n= 30)
0 (no response)	15.38^{abc}(4)	14.29^{abc} (7)	13.33^{abc}(4)
1 (slight response)	38.46^{ab} (10)	26.53 ^{ab} (13)	56.67 ^{c} (17)
2 (complete response)	46.15 ^{ab}(12)	59.18 ^{ab} (29)	30.00 ^{c} (9)

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA .
Values represent mean ± SE

Table (7) : Effect of $Cdcl_2$ on balance of neonates (% of neonates).

Items / groups	Control(n=41)	Low(n=18)	High(n=18)
No. of animals that fell From the rim.	9.76 ^{ab}(4)	11.11^{ab} (2)	44.44^{c} (8)

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA .
Values represent mean ± SEM.

Table (8): Effect of Cadmium on the behavior of rats in the open field test.

Items / groups	Control	Low	High
No.of squares crossed	26.87±6.71^{ab}	28.29±6.95^{ab}	58.00±7.21^{c}
No.of rears in the periphery.	05.13±1.03^{ab}	04.07±1.06^{ab}	11.00 ±1.10^{c}
Freezing (immobility time).(s).	1.00 ±0.85^{a}	3.20 ±2.48^{bc}	4.60 ±2.48^{bc}
No. of fecal boli.	2.61 ±0.50^{ab}	2.47 ±0.55^{ab}	4.00 ±0.64^{c}

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA .
Values represent mean ± SEM.

Table (9): Effect of Cadmium on the behavior of rats during the elevated plus maze test.

Items / groups	Control	Low	High
No.of entries(open arm)	11.00±3.13^{a}	5.15±3.01^{bc}	3.20±2.80^{bc}
Time spent (open arm)(s)	123.62±15.26^{ab}	117.15±16.39^{ab}	64.25±17.06^{c}
No.of entries(closed arm)	3.62±0.57^{abc}	5.92±0.57^{abc}	6.52±0.46^{abc}
Time spent (closed arm)(s)	60.46±12.20^{a}	108.77±12.20^{bc}	146.85±9.30^{bc}

a-c values within row with unlike superscripts differ significantly ($p < 0.05$) ; according to ANOVA .
Values represent mean ± SEM.

4. Discussion:

Studies in rodents have shown that, exposure to certain metal levels during gestation can cause maternal and developmental toxicity (Domingo et al; 2004). Our study, observed marked decline in birth weight of pups exposed to high doses of cadmium chloride in perinatal period. A significant retardation of intrauterine development manifested by lower body weight was reported in former study with female rats orally treated with $Cdcl_2$ (Barański, 1984). World Health Organization (WHO) (2001) confirmed that birth weights of newborn infants may be lower following maternal cadmium exposure. Unlike birth weights, our results reported no differences in body weight at weaning between control and experimental groups .Similar results derived from other studies with cadmium treated animals (Antonio et al., 2002). Also,Kierstin (2003) recorded no significant differences in body weights between the groups of pups prenatally exposed to cadmium .

Regarding physical maturation, the results showed delay in incisor eruption of pups exposed prenatally to high concentration of cadmium. Moreover, the data reported here revealed a negative effect of high concentration of $Cdcl_2$ on sexual maturation in terms of increased number of days at testes descent and vagina opening. In addition, no significant differences on pinna detachment or on the number of days at eye opening or fur growth were observed.

The results of the present work revealed that prenatal cadmium exposure produces a delay in neuromotor and neurobeavioural development of neonates (neonatal reflexes) in terms of righting reflex, negative geotaxis, cliff avoidance, startle responses and vibrissa reflex. In agreement with our results, Environment Agency (EA) (2009) reported that offspring from female rats exposed to 0.02-0.04 mg kg-1 bw day-1 prior to and during gestation showed impaired reflexes.

Regarding balance and coordination, impairment of rat's motor balance after ingestion of $Cdcl_2$ has been proven in the present study. Cadmium treated group exhibited the highest number of animals that fell from the rim of beaker. These results are consistent with former studies in rats (Ali et al., 1990; Hans, 2006; Ka-oud et al., 2010). Further support for impaired motor balance derived from increased symptoms of fatigue and disturbance of sensory & motor function (Murphy, 1997).

The current reduced birth weights & delay of some parameters of physical maturation and reflexes of neonates exposed prenatally and postnatal to high doses of cadmium might be explained on the basis of $Cdcl_2$ –induced adverse effects on serotonin, controlling body weight and maturation of reflex responses during the perinatal period in rats. Experimental evidence indicates that serotonin can influence embryogenesis and growth (Palén et al., 1979; Whitaker-Azmitia 1991). Furthermore, serotonin seems to play a role in regulating the development of the mammalian brain through actions on the serotoninergic neurons (Whitaker-Azmitia & Azmitia 1986; Shemer et al., 1991; Whitaker-Azmitia 1991).Also, Aghajanian and Marek (1997) reported the serotonin as a neurotransmitter has modulating effects on the neural excitability. Further support derived from earlier rat study for Teresa Cristina et al., (2008), where increased brain serotonin in young animals modulates their neuro- behavioural responses and growth.

The serotonergic system was found to be the most susceptible transmitter system in developing brain after cadmium exposure. Serotonin (5-HT) and its metabolite 5- hydroxyindoleacetic acid in cerebral cortex were reduced in pups exposed during suckling or during both the suckling and post weaning period (Kierstin, 2003). Confirmatory results derived from other study for Leret et al., (2003), where maternal co-exposure to lead and cadmium produced alterations in serotoninergic & dopaminergic systems of hippocampus.

The results showed that exposure to cadmium during the gestational & lactation period delayed the birth wight of young and the development of early behavior expression. Morphological or functional alterations produced during the period of fast brain development, indicating the participation of the serotoninergic mechanisms in these events. These findings together with previously mentioned observations go hand in hand with and further confirm the serotonin importance for maturation of most reflex responses during the prenatal & postnatal period in rats.

As the Environment Agency (EA) (2009) reported that cadmium can be fetotoxic, the most sensitive indicator appearing to be neurobehavioural development, with effects being seen on locomotor activity at around 0.2 – 0.4 mg cadmium kg-1 bw day-1 . Open field activity monitoring provides a non-invasive method for an accurate and comprehensive assessment of the motor activities of rats. The number of line crossing is usually used as a measure of locomotor activity, with high frequencies of this behaviour indicating increased locomotion activities (Eisenhaver and Murphy, 1998).In this study and as a trial to dissociate between "general activity" and "exploration", ambulation was only related to horizontal locomotion (amount of distance traveled) than vertical activity (rearing) which is more sensitive to anxiety state of the individual (Lapin et al., 1995; Brown et al., 1999). In the present work , increased

locomotor activity was noted in cadmium – exposed rats in the open field test .Animal studies with cadmium have shown that exposure leads to motor hyperactivity (Rastogi et al , 1977, **Wong and Klaassen** 1982). Also, in accordance with our data, Antonio et al., (2002) have observed alterations in motor activity in rats intoxicated with cadmium. Data reported in our previous article (Ka-oud et al., 2010), confirmed this justification, where cadmium-treated rats exhibited higher levels of activity upon exposure to open field test as revealed in enhanced line crossing. In contrast to our results Desi et al ,.(1998) demonstrated that cadmium treatment during prenatal development and the 4-week suckling period resulted in a significant dose-dependent decrease of horizontal and vertical exploratory activity and a significantly lower exploration frequency of the open-field centre. This discrepancy in results might be attributable to the different inoculated doses. Here, the enhancing effect of cadmium on locomotor activity might be explainable on the basis of endogenous levels of norepinephrine, dopamine and serotonin in various brain regions of cadmium-treated rats. Repeated daily intraperitoneal (i.p.) administrations of cadmium ($CdCl_2$, 1 mg/kg per day for 5 days) increased striatal dopamine (DA) release in 13-day-old rats (Elsa et al., 1998).

Anxiety in rats can be measured by behavioral reactivity to non-social or social stressors (Kim et al., 2004). These behaviors were compared by performing the open-field and elevated plus maze tests (non-social). With regard to the present study, it is important to note that most of the behavioural models cited above have mainly been used in the studies on the neurobiological mechanisms implicated in the production of fear and anxiety elicited in animals exposed to aversive situations (Rodgers and Dalvi, 1997; Menard and Treit, 1999).

The present work revealed that cadmium chloride caused a significant increase in the anxiety levels of rats in both of anxiety models used. Few researches have implemented open field test to investigate $Cdcl_2$ influence on anxiety levels in rats .Measures of anxiety in the open field test; number of rearing against the wall, freezing (immobility) as well as number of fecal boil, all parameters were greatly influenced by high dose of cadmium. Increased rearing behavior in the periphery has been proved to reflect higher levels of anxiety in rats (Anderson and Hughes, 2008). Supporting evidence for highly anxious rats in the current study derived from increased freezing time (immobility).Freezing has been validated as indicator of anxiety (Kalueff & Tuohimmaa, 2004). Where fecal boli were shown to be a sensitive measure for anxiety state of animals (Singer et al., 2005), toxicity with cadmium revealed enhance in defecation.

Also, data derived from elevated plus maze further affirmed the previously observed effect of cadmium on anxiety-related behaviours during open field test. Currently, data of elevated plus maze test revealed that cadmium treated animals exhibited low number of visits for open arms in EPM. Moreover, the less time was observed with high dose-administered rats indicating that they avoid this aversive region of the maze as reported in other studies (Bhattacharya et al., 1995; Schulteis et al., 1998). Therefore, time elapsed in the open arms might be considered as the more sensitive index for anxiety than number of visits. Our results are in line with data of (Bull, 2010) who stated that sub chronic oral exposure to cadmium can cause anxiety and alterations in the biochemical activity of the brain in laboratory animals .Also, Leret *et al*. (2003) recorded that, the intoxicated rats with cadmium and lead acetate, showed an increase on indices of anxiety on the elevated plus maze. These long –term changes in anxiety – like behavior can be related to dopaminergic and serotoninergic alterations detected in hippocampus. Where serotonin system is important in the pathophysiology of psychiatric disorders including mood and anxiety, healthy levels of serotonin is essential to promote balanced mood (Millan, 2003; Dayan and Huys, 2008). The hippocampal serotonergic alterations have been reported to play an important role in control of anxiety, depression and other mood disorders (File et al., 1996, 2000). Thus, the results indicate that neurochemical and neurobehavioural effects during development may be a more sensitive target for cadmium toxicity in animals models. Taken together, this study suggested that cadmium intoxication in gestational and lactation stage has negative potential effect on neuromotor maturation and behavioural development of neonates. Since maternal cadmium ingestion constitutes a great threat to progeny, caution should be exercised when products containing cadmium are administered to nursing mothers.

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References

Aghajanian, G.K., Marek, G.J.(1997): Serotonin induces excitatory postsynaptic potentials in apical dendrites of neocortical pyramidal cells. Neuropharmacology; 36:589-599.

Ali, M. M., Mathur, N., Chanra, S.V. (1990): Effect of chronic cadmium exposure on locomotor behavior of rats. Indian J Exp Biol. Jul; 28(7) :653- 6.

Anderson, N., Hughes, R. (2008): Increased emotional reactivity in rats following exposure to caffeine during adolescence. Neurotoxicol Teratol, 30: 195-201.

Antonio, M.T, Benito, M.J, Leret M.L, Corpas, I . (1998): Gestational administration of cadmium alters the neurotransmitter levels in newborn rat brains. J Appl Toxicol 18(2):83-88.

Antonio, M.T., Lo'pez , N., Leret, M.L. (2002): Pb and Cd poisoning during development alters cerebellar and striatal function in rats . Toxicology 176 , pp.59-66.

Antonio,S.M., Antonio,M.T.(2003): Perinatal exposure to lead and cadmium affects anxiety – like behavior . Toxicology,186, 125 -130.

Barański B. (1984) : Effect of exposure of pregnant rats to cadmium on prenatal and postnatal development of the young. J Hyg Epidemiol Microbiol Immunol. ;29(3):253-62.

Bhattacharya, S., Chakrabarti, A., Sandler, M., Glover, V. (1995): Rat brain monoamine oxidase A and B inhibitory (tribulin) activity during drug withdrawal anxiety. Neurosci Lett, 199: 103-106.

Brown, R., Corey, S., Moore, A. (1999): Differences in measures of exploration and fear in MHC-congenic C57BL/6J and B6-H-2K mice. Behav Gen 29(4): 263-271.

Bull, S. (2010): Cadmium; Toxicological Overview CHAPD HQ HPA – version: page, 1 -15.

Dayan, P., Huys, Q. (2008): Serotonin, inhibition, and negative mood. PLoS Comput Biol 4(2): e4. doi:10.1371/journal.pcbi.0040004

Desi,L., Nagymajte'nyi ,H. Schulz (1998) : Behavioural and neurotoxicological changes caused by cadmium treatment of rats during development . Journal of Applied Toxicology. Volume18 (1) 63-70.

Domingo,J.L.,Domingo,A.,Colomina,M.T.(2004): Influence of maternal stress on metal – induced pre- and postnatal effects in mammals :a review,Biol.Trace Elem.Res.98,193-208.

Eisenhaver, L., Murphy, M. (1998):Drug therapy and Physical assessment. In: Pharmacotherapeutics and advanced Nursing practice. NY: Mc Graw Hill, pp. 1-2.

Elsa ,Y. G., Arnulfo , A., and Camilo R. (1998): Increase of striatal dopamine release by cadmium in nursing rats and its prevention by dexamethasone-induced metallothionein. Toxicology Volume, 145-154.

Environment Agency (EA) (2009): Contaminants in soil: Updated collation of toxicological data and intake values for humans. Cadmium. Science report: SC050021 / TOX 3. Environment Agency. Bristol, Uk.

File, S., Cheeta, S., Kenny, P. (2000): Neurobiological mechanisms by which nicotine mediates different types of anxiety. Eur J Pharmacol, 393(1-3): 231-236.

File, S.,Gonzalez, L., Andrews, N. (1996): Comparative study of pre- and postsynaptic 5-HT1A receptor modulation of anxiety in two ethological animal tests. J Neurosci, 16: 4810-4815.

Hans, W. (2006): The hazards of Cadmium in man and animals. Journal of applied toxicology, 2, 61-67.

Kalueff, A., Tuohimaa, P. (2004): Experimental modeling of anxiety and depression. Acta Neurobiol Exp, 64: 439-448 Neurobiol Exp, 64: 439-448.

Ka-oud H., Mervat M.K., Abeer H. A., Gehan M. K., and Kawkb A.A.(2010) : Neurobehavioural, neurochemical and neuromorphological effects of cadmium in male rats. Journal of American Science 6 (5) 189 -202.

Kelly, A. (1993): Locomotor activity and exploration. In: Sahgal, A. (Ed.), Behavioural Neuroscience vol. II: a practical approach. Oxford University Press, Oxford, pp. 1-21.

Kierstin, P.G. (2003): Lactational transfer of cadmium in rodents – CNS effects in the offspring. Doctoral thesis – Swedish University of Agricultural Sciences – Uppsala

Kim, S., Kang, E., Park, C. (2004): Comparative analysis of the responses to intruders with anxiety-related behaviors of mouse. Kor J Biolog Sci, 8(4): 310-306.

Lapin, I., Khaunina, R., Mirzaev, S. (1995): Vertical motor activity of mice is slowed by lower doses of psychotropic drugs than horizontal. Bull Eksp Biol Med, 120: 385-387.

Leret ,ML., Millán, JA., Antonio MT.(2003): Perinatal exposure to lead and cadmium affects anxiety-like behaviour. Toxicology. 15; 186(1-2):125-30.

Meer,P.V. , Raber,J (2005) : Mouse behavioural analysis in systems biology. Biochem.J.389 (593-610).

Menard, J., Treit, D. (1999): Effects of centrally administered anxiolytic compounds in animal models of anxiety. Neurosci Biobehav Rev, 23: 591-613.

Millan, M. (2003): The neurobiology and control of anxious states. Prog Neurobiol, 70: 83-244.

Murphy , V .A. (1997): Cadmium : Acute and chronic neurological disorders . In : Yasui M , Strong M ,Ota K, Verity MA (Eds).Mineral and

Metal Neurotoxicology. Boca Raton , Fl :CRC press, pp. 229- 240.

Palén,K., Thörneby, L., Emanuelsson., H. (1979) : Effects of serotonin antagonists on chick embryogenesis. Wilhelm Roux's. Arch Dev Biol; 187:89-103.

Rastogi, R.B., Merali, z., Singhal,R.L. (1977): Cadmium alters behaviour and the biosynthetic capacity for catecholamines and serotonin in neonatal brains . Journal of Neurochemistry, 28, 789 – 794.

Rice,D.,Barone ,Jr,S.(2000) : Critical periods of vulnerability for the developing nervous systems: evidence from humans and animal models .Environmental Health Perspectives 108(suppl.3),511-53.

Rodgers, R., Dalvi, A. (1997): Anxiety, defense and the elevated plus-maze. Neurosci Biobehav Rev, 21: 801-810.

Schulteis, G., Yackey, M., Risbrough,V., Koob,G. (1998): Anxiogenic-like effects of spontaneous and naloxone-precipitated opiate withdrawal in the elevated plus maze. Pharmacol Biochem Behav, 60: 727-31.

Shemer, A.V., Azmitia, E.C., Whitaker-Azmitia, P.M. (1991): Dose-related effects of prenatal 5-methoxytryptamine (5-MT) on development of serotonin terminal density and behavior. Dev. Brain Res; 59:59-63.

Singer, J., Hill, A., Nadeau, J., and Lander, E. (2005): Mapping quantitative trait loci for anxiety in chromosome substitution strains of mice. Gen, 169(2): 855-862.

Smart,J.L.,Dobbing,J.(1971):Vulnerability of developing brain .II. Effects of early nutritional deprivation on reflex ontogeny and development of behavior in the rat. Brain Research, 28;85-95.

SPSS (2006): SPSS for Windows 14.0.

Tanaka,T. (2006): Reproductive and neurobehavioural toxicity study of tartrazine administered to mice in the diet. Food Chem Toxicol, 44(2): 179-187.

Teresa ,C.B.de Jesus D., Judelita, C., Elizabeth do Nascimento, Jaiza ,M.B.M., Fabiana ,C., Kelli, N.F., Raul, M. de- Castro,(2008) : Neonatal exposure to Citalopram, a serotonin selective reuptake inhibitor , programs a delay in the reflex ontogeny in rats.Arq.Neuro-Psiquiatr.vol.66 no.3b Sa'o Paulo.

Trullas, R., Skolnick, P. (1993): Differences in fear motivated behaviours among inbred mouse strains. Psychopharmacol., 111: 323-331.

Waalkes , M.P., Anver , M. R., Diwan, B.A.(1999) : Chronic toxic and carcinogenic effects of oral cadmium in the Noble (NBL . / Cr): induction of neoplastic and proliferative lesions of the adrenal , kidney . Prostate and testes. J.Toxicol. Environ. Health A . 58, 199- 214.

Walf, A., Frye, C. (2007): The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc, 2(2): 322-328.

Whitaker-Azmitia PM (1991):PM. Role of serotonin and other neurotransmitter receptors in brain development: basis for developmental pharmacology. Pharmacol Rev; 43:553-561.

Whitaker-Azmitia PM, Azmitia EC. (1986): Autoregulation of fetal serotonergic neuronal development: role of high affinity serotonin receptors. Neurosci Letters; 67:307-312.

Wong, K., Klaassen, C. D. (1982): Neurotoxic effects of cadmium in young rats. Pharmacology Volume, May 1982, Pages 330-337

World Health Organization (WHO) (2001): Safety evaluation of certain food additives and contaminants. Fifty-fifth meeting of the joint FAO/WHO expert committee on feed additives, Toxicological monographs, WHO food additives series No 46. WHO. Geneva.

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The Moderating Role of Training on the Relationship between Transformational Leadership and Creativity

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Abstract: Although research studies have indicated that transformational leadership style is related to creativity, relatively limited researches have attempted to determine the moderating effect of training on the relationship between transformational leadership and creativity. Data were collected from 110 postgraduate students at the Faculty of Education of research universities in Malaysia. In this study, Moderated Multiple Regression analysis was used to test hypothesis and theoretical model. Findings of this study indicated that training factor (untrained students vs. trained students) significantly affected the relationship between transformational leadership and creativity. It is suggested that supervisors should engage in transformational leadership behaviors in order to enhance the creative performance of their students. Also, creativity training program should be provided for students to learn creativity knowledge, attitudes, workplace idea generation and idea implementation.
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Keywords: Transformational leadership; creativity; training; moderated multiple regression.

1. Introduction

Creativity is a critical factor to the success and competitive advantage of educational organizations (Gumusluoglu & Ilsev, 2009). Innovation and global competition has motivated educational organizations to exert creativity to improve students and increase efficiency and effectiveness of their organizations. Many researchers believed that leadership is the significant factor in creating creativity in organization (Mumford, Scott, Gaddis, & Strange, 2002; Shin & Zhou, 2003). One of the styles of leadership that can facilitate the generating and implementing new ideas within organization is transformational leadership style (Andriopoulos & Lowe, 2000). Also, Scott, Leritz and Mumford (2004) emphasized that training can enhance creativity. It would seem that training may have an impact on the relationship between leadership and creativity. However, limited studies have been conducted regarding the moderation role of training on the relationship between transformational leadership and creativity. In this paper, we are interested to investigate the effect of transformational leadership behaviours of supervisors on their students' creativity. Training factor (trained students / untrained students) is hypothesized as a moderator variable in an effort to understand which group of students has a stronger impact on the relationship between these two variables.

H_1: Training (untrained / trained groups) has a significant moderating effect on the relationship between transformational leadership and creativity.

This article will provide some preliminary findings of a study currently being conducted in Malaysian research universities. The remainder of this article is organized as follows. In Section 2, we introduce the relevant literature; Section 3 describes methodology and is followed by presentation of the findings. Finally, we describe a summary and discussion for future research.

2. Leadership and Creativity

According to Gumusluoglu and Ilsev (2009) "creativity is the production of novel and useful ideas" (p. 461). It is an important factor for organizations' survival and competitiveness (Gong, Huang & Farh, 2009). Also, it has significant impact students' performance. Some research studies have reported that students' creativity will be flourished when their lecturer or supervisor displays transformational leadership behaviours (Gong, Huang & Farh, 2009). Transformational leaders create changes within organizations. It refers to the process whereby a leader engages with his followers and creates a connection that raises the level of motivation and morality in both himself and his followers (Northouse, 2009, p. 131). In fact, this type of leadership is becoming more and more important to organizations, as workforces become more diverse, technology improves and international competition heightens (Afshari et al., 2010).

In 2009, Gumusluoglu and Ilsew conducted a study on transformational leadership and creativity and found that "transformational leadership behaviors closely match the determinants of innovation and creativity at the workplace, some of which are vision,

support for innovation, autonomy, encouragement, recognition, and challenge" (P.462). In fact, these behaviors are as instrumental for promoting creativity (Sosik, Kahai, & Avolio, 1998). Similarly, Gong et al. (2009) found that transformational leadership style of managers was positively related to employees' creativity.

Moreover, Dvir and his colleagues (2002) conducted a study on impact of transformational leadership on follower development and found that there is a significant relationship between transformational leadership role of teachers and students' empowerment. In fact, students under transformational leadership are encouraged to seek creative approaches in completing their tasks and feel empowered. Empowerment is a source of creativity. People who are empowered can show creative behavior (Jung, et al., 2003).

According to Gumusluoglu and Ilsev (2009), "transformational leaders, by individualized consideration, can enhance self-confidence of their followers and heighten personal development that leads to follower empowerment" (p.463). Transformational leader can establish a pleasant environment and stimulate followers to be creative and innovative. Also, "they can encourage followers to try new approaches and develop innovative ways of dealing with organizational issues" (Northouse, 2009, p. 142). Furthermore, theses leaders by "intellectually stimulating their followers, championing innovation, and articulating a compelling vision throughout their organizations, help establish an organizational climate where employees feel challenged and energized to seek innovative approaches in their jobs" (Koene, Vogelaar, & Soeters, 2002 cited in Gumusluoglu & Ilsev, 2009). It would seem that teachers who act as transformational leaders can create an open, flexible, unconventional, and student-centered environment (Andiliou & Murphy, 2010). In such environment, personality characteristics, thinking styles, knowledge, and skills needed for creative thinking will be developed (Andiliou & Murphy, 2010).

3. Training and creativity

A number of studies indicated that training can foster creativity. The traditional belief is that creative people are geniuses, working on creative endeavours in isolation from the rest of the world. Andriopoulos and Dawson (2009) believed that everyone can be creative so training should be provided for individuals to understand the process of creativity. In fact, 'through education and training the innate creative ability of individuals can be stimulated and nourished' (Rose and Lin, 1992:131). This is supported by De Bono (2004) who studied on creativity training programs and found that training of a creative thinking process can enhance creativity.

Amabile (2001) introduced Componential model for creativity as a way of training creativity. According to this model, individuals' cognitive abilities (mental flexibility, remote associations, suspension of judgement, and originality of thinking), personality traits (risk taking, self-confidence, need for achievement, autonomy), knowledge and motivation to innovate will be fostered. Moreover, Cave (1999) introduced two different approaches for training creativity: 1) to motivate and inspire students in order to be creative; 2) to teach them the cognitive techniques to generate new ideas. Furthermore, Basadur, Runco and Vega (2000) reported that creativity training enhanced generating more, higher-quality and more original ideas. It would seem that educational institutions should invest substantial time and resources and provide training programs for staff to learn creativity knowledge, attitudes, workplace idea generation and idea implementation (Birdi, 2005).

3. Methodology

The moderating effect of training between transformational leadership and creativity was analyzed using moderated multiple regression (MMR) analysis. First, preliminary analyses such as normality, linearity, homoscedasticity, and homogeneity of error variance were tested before running the MMR analysis.

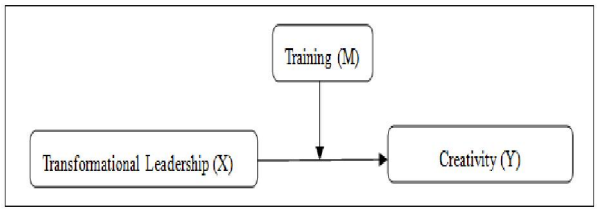

Figure1. Simple research framework

The equation one shows the variables in the ordinary least-square (OLS) model.

Equation 1 (OLS model): $Y= \beta_0 + \beta_1 X + \beta_2 Z + e$

Also, equation 2 compares the OLS model with the MMR model to identify the presence of moderating effect.

Equation 2 (MMR model): $Y= \beta_0 + \beta_1 X + \beta_2 Z + \beta_3 X*Z + e$

"Where Y= creativity, X= transformational leadership, Z= training , X*Z= the product between the predictors transformational leadership and training (TL * Training), β_0= the intercept of the line-of-best-of-fit which represents the value of y when X=0, β_1 = the least-squares estimate of the population regression coefficient for X, β_2= the least-squares

estimate of the population regression coefficient for Z, β_3= the sample- base least-squares estimates of the population regression coefficient for the product term, and e= the error term" (Aguinis, 2004, p.178).

Quantitative data were gathered through a set of questionnaire. Transformational leadership style was measured by the Multifactor Leadership Questionnaire. It consists of 20 items. Also, creativity consists of 13 items measuring students' creativity. The moderator variable (training) was measured based on nominal scale (trained students and untrained students). Face and content validity of these instruments were established by a panel of experts. Feedback from the panel of experts was used to ensure that these scales measure the content areas of investigation and are culturally and technically appropriate for the context of the study. In addition, Cronbach's alpha was used to measure internal consistency and calculated via the SPSS 18 statistical package. Cronbach's alpha is the most common form of internal consistency reliability coefficient. The Cronbach's alpha coefficients for these scales were: transformational leadership style = .913 and creativity= .85.

Table 2. Coefficient

Coefficients								
		Unstandardized coefficients		Standardized coefficients			95% confidence interval for B	
	Model	B	Std.Error	Beta	t	Sig.	Lower bound	Upper bound
1	Constant:	2.408	0.308		7.813	0.000	1.797	3.019
	Transformational leadership	0.320	0.085	0.291	3.763	0.000	0.151	0.489
	Trained/Untrained	0.367	0.057	0.495	6.404	0.000	0.253	0.480
2	Constant:	1.595	0.437		3.654	0.000	0.730	2.461
	Transformational leadership	0.546	0.121	0.496	4.513	0.000	0.306	0.785
	Trained / Untrained	1.928	0.611	2.601	3.156	0.000	0.717	3.140
	TL* Training	-0.021	0.008	-2.168	-2.566	0.002	-0.038	-0.005

Table 1. Model summary

Model Summary									
					Change statistics				
Model	R	R^2	Adjusted R^2	Std. error of the estimate	R^2 Change	F change	Df1	Df2	Sig. F change
1	0.626	0.392	0.381	0.293	0.392	34.507	2	107	0.000
2	0.654	0.428	0.411	0.286	0.036	6.585	1	106	0.002

According to Model 1 of the result of MMR analysis, R= 0.626, R^2= 0.392, F (2, 107) = 34.507, and P=0.0001. This result shows that 39.2% (R^2= 0.392) of the variance in creativity is explained by transformational leadership style and training (Table 1). Moreover, based on Table 2, the regression equation for model 1 is:

Equation 1: Creativity = 2.408 + 0.320 transformational leadership + 0.367 training

The regression equation1 shows that for every one unit increase in transformational leadership, the level of creativity will increase by 0.320 units and for every one unit increase in training, the level of creativity will increase by 0.367 units.

Model 2 indicates the results after the product term (TL*Training) was included in the equation. As can be seen from Table 1, the addition of the product term resulted in an R^2 change of 0.036, [F (1, 106) =6.585, P<0.05]. The results support for the presence of a moderating effect. In other word, the moderating effect of training explains 36% of variance in creativity increase above and beyond the variance explained by transformational leadership scores and training. So, it can be concluded that the hypothesis is supported.

The equation for model 2 is as follows:

Equation 2: creativity= 1.595 + 0.546 transformational leadership + 1.928 Training – 0.021 TL*Training

In fact, dummy coding system was used to code the binary moderated. The equation above shows that there is -0.021 differences between the slop of creativity increase on transformational leadership between untrained students (coded as 0) and the trained students (coded as 1). In other words, the slop regression creativity on transformational leadership is less steep for untrained students as compared to trained students.

Predicted Creativity = 1.595 + 0.546 transformational leadership + 1.928 Training – 0.021 TL*training

Predicted Creativity = 1.595 + 0.546 transformational leadership + 1.928 (1) – 0.021 TL*(1)

Equation 3: untrained students predicted creativity = 1.595 + 0.546 transformational leadership

Equation 4: trained students predicted creativity = 3.523+0.525 transformational leadership

Also, value of 1 standard deviation (SD) above and below the mean for transformational leadership was selected. Mean score for transformational leadership is 3.67 and the standard deviation is 1.03. By using the value of 2.64 (1 SD below mean) and 4.7 (1SD above mean) Equation 3 and 4 yield the graph shown in Figure 2:

Y= 3.523+0.525 (2.64) = 4.90
Y=3.523+0.525 (4.008) = 6.00
Y= 1.595 +0.546 (2.64) = 3.04
Y= 1.595+0.546 (4.008) =4.1

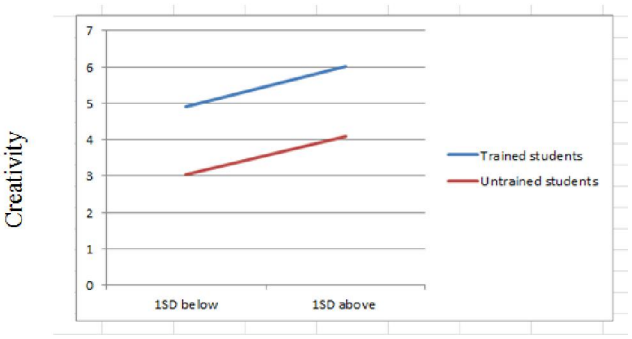

Figure 2. Slop for transformational leadership and creativity on training

3. Conclusion

The Research studies showed the important role of transformational leadership on creativity. These studies indicated that there is a significant positive relationship between transformational leadership and creativity. However, findings of this study indicated that creativity training play a significant role in moderating the relationship between transformational leadership and creativity. In other words, the relationship between transformational leadership and creativity is stronger for students who attended creativity workshop or training. This might be due to the fact that training can raise each student's creative potential by developing of knowledge, skills and attributes related with creativity. Moreover, this result indicated that students who have attended creative training programs and their supervisors encourage them to be creative through intrinsic motivation, empowerment, and provide supportive climate for innovation; their level of creativity will be higher. It is suggested that supervisors should engage in transformational leadership behaviors (charisma, intellectual stimulation, inspirational motivation, and individual consideration) in order to enhance the creative performance of their students. Also, creativity training program should be provided for students to raise their ability to generate ideas and solve problems.

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References

1. Afshari M, Abu Bakar K, Luan WS, Afshari M, Fooi FS, Abu Samah B. Computer use by secondary school principals. Turkish Online Journal of Educational Technology 2010; 9(3): 8-25.
2. Agunis H. Regression analysis for categorical moderators. London, New York, 2004.
3. Amabile TM. Beyond talent. American psychologist 2001; 56(4): 333-337.
4. Andiliou A, Murphy K. Examining variations among researchers' and teachers' conceptualizations of creativity: A review and synthesis of contemporary research. Educational Research Review 2010; 5 (3): 210-219.
5. Azizi J, Taimoori S R, Papzan A, Allahyari M. The Impacts of Globalization on Rural Communities of Kermanshah Township, Iran. Life Science Journal 2011; 8(1):13–19.
6. Andriopoulos C, Lowe A. Enhancing organisational creativity: the process of perpetual challenging. Management Decision 2000; 38(10):734-742.
7. Basadur M, Runco MA, Vega LA. Understanding how creative thinking skills, attitudes and behaviors work together: a causal process model. Journal of creative behaviour 2000; 34(2):77-100.
8. Birid KS. No idea? Evaluating the effectiveness of creativity training. Journal of European industrial training 2005; 29(2): 102-111.
9. Cave C. Definitions of creativity 1999. http://members.optusnet.com.au/~charles57/Creative/Basics/definitions.htm. Access: 5 February 2011.
10. De Bono E. Discussion on lateral thinking techniques 2004. http://www.edwarddebono.com. Access: 12 May 2011.
11. Dvir T. Eden D, Avolio B J, Shamir B. Impact of transformational leadership on follower development and performance: A field experiment. The Academy of Management Journal 2002; 45(4): 735-744.
12. Gong Y, Huang JC, Farh, J L. Employee learning orientation, transformational leadership, and employee creativity: The mediating role of employee creative self-efficacy. Academy of Management Journal 2009; 52 (2): 123-150.
13. Gumusluoglu L, Ilsev A. Transformational leadership, creativity, and organizational innovation. Journal of Business Research 2009; 62(4): 461-473.
14. Jung DI, Chow C, Wu A. The role of transformational leadership in enhancing organizational innovation: Hypotheses and some preliminary findings. The Leadership Quarterly 2003; 14(4-5): 525-544.
15. Mumford MD, Scott G M, Gaddis B, Strange JM. Leading creative people: Orchestrating

expertise and relationships. The Leadership Quarterly 2002; 13(6): 705-750.
16. Northouse PG. Leadership: Theory and practice. Thousand Oaks, CA: Sage Publications, 2009.
17. Rose LH, Lin H. A meta-analysis of long-term creativity programs. Journal of creative behavior 1992; l8(l):124-131.
18. Shin SJ, Zhou J. Transformational leadership, conservation, and creativity: Evidence from Korea. The Academy of Management Journal 2003;46(6): 703-714.
19. Scott G, Leritz L, Mumford M. The effectiveness of creativity training: A quantitative review. Creativity Research Journal 2004; 10 (2):151-158.
20. Sosik JJ, Kahai SS, Avolio BJ. Transformational leadership and dimensions of creativity: Motivating idea generation in computer-mediated groups. Creativity Research Journal 1998; 10(3): 55-61.

11/11/2011

Effect of Abdominal Versus Pelvic Floor Muscles Exercises on Vaginal and Leak Point Pressures in Mild Stress Urinary Incontinence in Obese Women

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Abstract: Objective: To compare the benefits of 12 weeks abdominal and pelvic floor muscles (PFM) strength training for mild stress urinary incontinence (SUI) in obese women. **Design**: A randomized control trial with three months follow up. **Setting**: Bab El Sharia University Hospital. **Subjects**: Thirty female obese patients with mild SUI. **Intervention**: Abdominal exercises (Abd. ex's) group (n=15) received specific exercises for transversus abdominis and internal obliqus muscles. Whereas, pelvic floor exercises (PF ex's) group (n=15) received pelvic floor exercises. **Main outcome measures**: Vaginal pressure, leak point pressure (LPP) and waist hip ratio (WHR) were measured for both groups at three intervals (baseline, 12 weeks of intervention and 3months follow up i.e. 24 weeks from the start of the study). **Results**: Both abdominal and pelvic floor groups showed a significant increase in vaginal pressure after 12 weeks of intervention ($p < 0.0001$ and $p < 0.021$, respectively) and at follow up ($p < 0.0001$ and $p < 0.009$, respectively) compared to baseline. This effect was greater for Abd. ex's group at 12 weeks ($p < 0.041$) and at follow up ($p < 0.022$) when compared with PF ex's group. Also, both abdominal and pelvic floor groups showed a significant increase in LPP after 12 weeks of treatment ($p < 0.001$ and $p < 0.008$, respectively) and at follow up ($p < 0.0001$ and $p < 0.007$, respectively) compared to baseline; there were no significant differences between the two groups at these time points. **Conclusion**: Overall, the results of this study suggest that 12 weeks of abdominal muscles strengthening training has superior effects compared to pelvic floor strength training for mild SUI in obese patients.
[Dalia M. Kamel Ali A. Thabet Sayed A. Tantawy and [3]Mohamed M. Radwan **Effect of Abdominal Versus Pelvic Floor Muscles Exercises on Vaginal and Leak Point Pressures in Mild Stress Urinary Incontinence in Obese Women]** Life Science Journal,. 2011; 8(4):542-549] (ISSN: 1097-8135).
http://www.lifesciencesite.com.

Keywords: Urinary incontinence; obesity; pelvic floor; abdominal; exercise.

1. Introduction

Stress urinary incontinence (SUI) is urodynamically proved as involuntary loss of urine occurs following a sudden rise in the intra-abdominal pressure caused by coughing, sneezing, straining, laughing or other physical activities, when the intravesical pressure exceeds the maximum urethral pressure in the absence of detrusor contraction [1, 2]. SUI is the most common type of urinary incontinence in women with risk factors includes advancing age, childbirth, smoking, chronic bronchitis, and obesity [3].

There are many methods to diagnose SUI. Leak point pressure (LPP) testing originated from extensive video urodynamic studies done over many years in a board cross-section of patients including those with idiopathic incontinence, stress incontinence and neurogenic conditions [4]. In addition, the perineometer, through a compressible vaginal catheter that is connected to a manometer, measures the increase of intravaginal pressure that is produced by the contraction of pelvic floor muscle [5].

Obesity has often been suggested as a risk factor for urinary incontinence. There are several mechanical and physiologic reasons why an increased body mass index (BMI) may be associated with, if not causative of, urinary incontinence [6]. Each 5-unit increase in BMI associated with a 60% to100% increased risk of daily incontinence [7]. So, there is a strong association between increasing weight and SUI as there is higher resting intra-abdominal and intravesical pressures in obese individuals [8]. Increased intra-abdominal pressures adversely stress the pelvic floor in addition to affect the neuromuscular function of the genitourinary tract [9].

The increase in intravesical pressure created by a rising BMI may reduce the continence gradient between the urethra and the bladder. In this situation, the magnitude of increased intra-abdominal pressure necessary to force urine through the urethra is reduced because the static pressure within the bladder is higher [10]. These higher pressures could expose the pelvic-floor muscles to a state of chronic stress, and place chronic stretch on the pudendal nerve [11].

Pelvic floor exercises [12, 13] are advised as a first line of treatment for female SUI. These exercises advocated to strength weak perineal and pelvic floor muscles but their success depend on high level of patients' motivation and compliance with an individual exercise program [14].

Contraction of the abdominal muscles may provide an efficient mechanism with which contraction of the pelvic floor muscles is initiated, particularly for patients who have difficulty in learning to contract those muscles, however the use of abdominal muscles training to rehabilitate pelvic floor muscles may be useful in treating SUI [15]. **Madill and McLean** [16] found that deep abdominal muscle contraction increased intra-vaginal pressure. Moreover, pelvic floor muscles act as part of an integrated abdominal-pelvic unite, under control of central nervous system programming that ensures appropriate timing of automatic responses to any change in trunk postures and trunk muscles activity [17]. The close association between the pelvic floor muscles and abdominal muscles comes from **Power** [18], who described a direct continuation of puborectalis with rectus abdominis in an imperfectly developed fetus [19].

Some studies indicated that abdominal activity and pelvic floor muscles contraction are a normal response to each other. The response of the abdominal muscles to voluntary contraction of the pelvic floor muscles showed greater electromyography (EMG) activity amplitude of transversus abdominis than that of rectus abdominis and obliquus externus abdominis when the spine was positioned in extension [20]**.** When specific isometric abdominal contractions were performed in lying position, pelvic floor contraction EMG activity increased. Also, urethral pressure increases with voluntary pelvic floor muscles contraction and isometric abdominal muscles holds [21, 22].

So far, only one randomized control trial has addressed the effect of abdominal muscle training on SUI. The results showed that additional training of the transversus abdominis (TrA) after pelvic floor muscle training (PFMT) and neuromuscular stimulation did not provide incremental improvement of SUI [23]. However, the coactivation and coordination of the TrA and PFM was not the target.

According to the previously mentioned facts, we encouraged to make an attempt to compare the response when training each of abdominal and pelvic floor muscles separately for mild SUI in obese women.

2. Patients and Methods

Thirty female patients were diagnosed with mild SUI. The diagnosis made via history taking, vaginal examination & Urodynamics study. The patients were referred from the gynecological and urological outpatients' clinics at Bab El Sharia University Hospital. The ethical committee in the hospital approved the study. Inclusion criteria were: age 30-40 years, parity $\leq$ 3 times, BMI 30-34 Kg/ m^2, and waist\hip ratio $\geq$ 0.8. Demographic data are summarized in table1. The exclusion criteria were pregnancy, lower urinary tract infections, neurological problems, pelvic tumor, diabetes, smoking, chronic chest diseases as well as, other types of urinary incontinence, and any medications or medical\surgical interventions for SUI.

All patients gave a written consent to participate in the study and were provided with a full explanation of the treatment protocol.

Assessment procedures

Patients were assessed at three time points: baseline, following 12 weeks of exercise intervention and then after 24 weeks from the beginning of the study as follow-up. Outcome measures were as follows:

Perineometer (Peritron 9300; Cardio Design Pty Ltd Australia) assess vaginal pressure as a marker of pelvic floor muscle strength. During assessment, the patients were asked to strongly squeeze, lift and maintain hold (as long as possible) on the vaginal probe of the perineometer. In addition, the patients taught not to involve rectus abdominus or the gluteal muscles at all during assessment. The examiner observed the cranial movement of the perineum through the slight anterior tilt of the sensor (towards the anus) and recorded of the readings over the monitor. This maneuver was repeated three times per session and the mean of vaginal pressure was calculated

Urodynamics studies were performed after the approval of ethical committee by using a Merkur 2000 in order to confirm the diagnosis of SUI and also to measure valsalva LPP.

Weight/height scale measurements were used to calculate the BMI, in order to confirm the degree of the patient's obesity. This was done only one time at baseline assessment.

Tape measurement was used to calculate waist/ hip ratio (which must be $\geq$ 0.8) at baseline assessment. The normal value is 0.7 for women [24].

Procedures

Eligible patients were randomly allocated into two groups by using simple random method. Concealed papers picked by a third parity to pick patient's name for each group at a time. By the end, there were two groups abdominal exercises (Abd. ex's) group (n=15) underwent abdominal muscles exercise strength training program specifically for TrA and internal oblique muscles [19]; and pelvic floor exercises (PF ex's) group (n=15) underwent pelvic floor muscles strength training program. The intervention was applied at the physiotherapy outpatient clinic in an isolated and secured place. Both groups trained for 12 weeks with frequency 3sessions/week (see Appendix for details).

All patients received the standard treatment for SUI and obesity including education,

advice and dietary modification in form of 1200 Kcals\ day divided into 3 main meals and 2 snacks in addition to counseling and diet modification every week during the intervention. Both groups were asked to continue their own program plus the dietary modification after the intervention until they reassessed after 3 months. Statistical analysis, applied the central limit theory that assuming large sample. Statistical comparisons within each group were made using paired t-test for pre and post treatment measurement variables. Comparisons between groups were made using unpaired t-test. The P-value was set at 5% level.

3. Results

Thirty female patients were recruited and randomized into two groups (Abd. ex's & PF ex's). There were no differences at the baseline between the groups in the age, weight, height and BMI as presented in table1.

Vaginal pressure, there were no significant differences between the groups at baseline. Both groups (Abd. ex's & PF ex's) showed a significant increase in vaginal pressure after 12 weeks of treatment ($p < 0.0001$ and $p < 0.021$ respectively) and after 24weeks ($p < 0.0001$and $p < 0.009$ respectively) compared to baseline, table2. When comparing both groups, the increased vaginal pressure was greater in Abd. ex's group than PF ex's group at 12 weeks ($p < 0.041$) and after 24 weeks ($p < 0.022$), Fig.1. The improvement percentages after 12 & 24 weeks were 15.620% &18.02%, respectively in Abd. ex's group, while in PF ex's group were 4.6% and 5.96% respectively.

Leak Point Pressure, there were no significant differences between the groups at baseline. Both groups (Abd. ex's & PF ex's) showed a highly significant increase after 12 weeks of treatment ($p < 0.001$ and $p < 0.008$, respectively) and after 24weeks ($p < 0.0001$ and $p < 0.007$, respectively) compared to baseline(Table3). Comparing both groups, there were no significant differences at 12 weeks ($p < 0.205$) & 24 weeks ($p < 0.058$), Fig.2. The improvement percentages after 12 & 24 weeks were 16 % and 16.83 %, respectively in Abd. ex's group. While there were (9.07% &7.66% respectively) in PF ex's group.

Waist/hip ratio, there were no significant differences between the groups at baseline. Both groups (Abd. ex's & PF ex's) showed a significant decrease in WHR after 12 weeks & after 24weeks compared to baseline Abd. ex's group: $p < 0.0001$ & $p < 0.0001$; and PF ex's group: $p < 0.021$ & $p < 0.006$, respectively(Table 4). In comparison of both groups, there were no significant differences at 12 weeks ($p < 0.095$) & 24 weeks ($p < 0.069$), Fig.3.

Table1 The demographic data of subjects in both groups.

	Groups	Range		Mean	SD	t-value	P- value
		Min.	Max.				
Age (Yrs)	Abd ex's group	35	45	39.87	3.54	0.11	0.92
	PF ex's group	35	45	39.74	3.64		
Weight (Kgs)	Abd ex's group	72	97	84.94	7.35	0.99	0.34
	PF ex's group	70	98	82.80	8.10		
Height (Cm)	Abd ex's group	153	174	162.27	7.86	1.04	0.32
	PF ex's group	152	173	160.20	7.36		
BMI (Kg/m^2)	Abd ex's group	30.72	33.75	32.23	0.93	0.12	0.91
	PF ex's group	31.11	33.75	32.20	0.83		

Table 2. The mean difference values of the vaginal pressure at baseline, post 12 & 24 weeks in both groups.

		Mean difference	S.D.	t-value	P-value	significance
Abd ex's group	Baseline Post 1	-7.80	5.89	-5.12	0.0001	Significant
	Baseline Post 2	-9.00	5.65	-6.16	0.0001	Significant
	Post1 Post 2	-1.20	3.50	-1.32	0.207	Non significant
PF ex's group	Baseline Post 1	-2.33	3.47	-2.59	0.021	Significant
	Baseline Post 2	-3.00	3.82	-3.04	0.009	Significant
	Post1 Post 2	-0.66	1.04	-2.46	0.027	Significant

Key S.D. = standard deviation Post 1= 12 weeks Post 2= 24 weeks

Table 3. The mean difference values of the LPP at baseline, post 12 & 24 weeks in both groups.

		Mean difference	S.D.	t-value	P-value	significance
Abd ex's group	Baseline Post 1	-12.80	11.44	-4.33	0.001	Significant
	Baseline Post 2	-13.46	9.87	-5.28	0.0001	Significant
	Post1 Post 2	-0.66	2.69	-0.96	0.353	Non significant
PF ex's group	Baseline Post 1	-7.26	9.18	-3.06	0.008	Significant
	Baseline Post 2	-6.13	7.47	-3.18	0.007	Significant
	Post1 Post 2	-1.13	5.01	0.87	0.396	Non significant

Table 4. The mean difference values of the WHR at baseline, post 12 & 24 weeks of in both groups.

		Mean difference	S.D.	t-value	P-value	significance
Abd ex's group	Baseline Post 1	0.048	0.037	4.990	0.0001	Significant
	Baseline Post 2	0.052	0.035	5.674	0.0001	Significant
	Post1 Post 2	0.003	0.024	0.529	0.605	Significant
PF ex's group	Baseline Post 1	0.018	0.026	2.60	0.021	Significant
	Baseline Post 2	0.022	0.027	3.238	0.006	Significant
	Post1 Post 2	0.004	0.013	1.38	0.187	Non significant

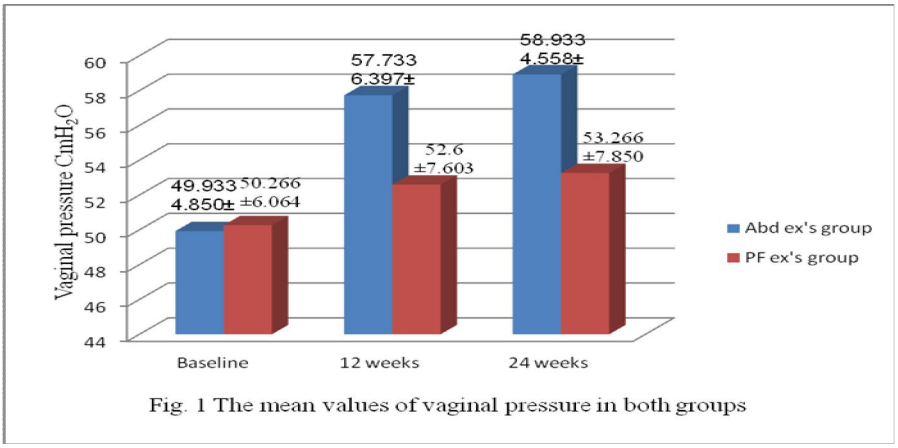

Fig. 1 The mean values of vaginal pressure in both groups

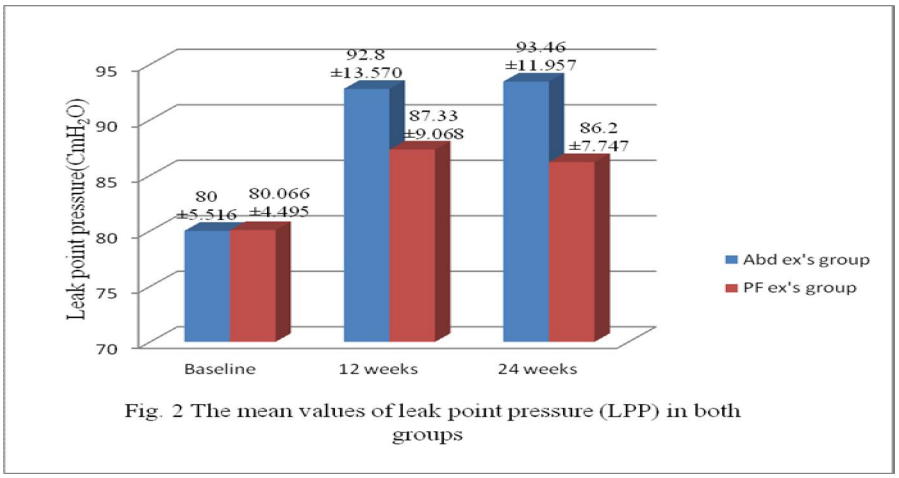

Fig. 2 The mean values of leak point pressure (LPP) in both groups

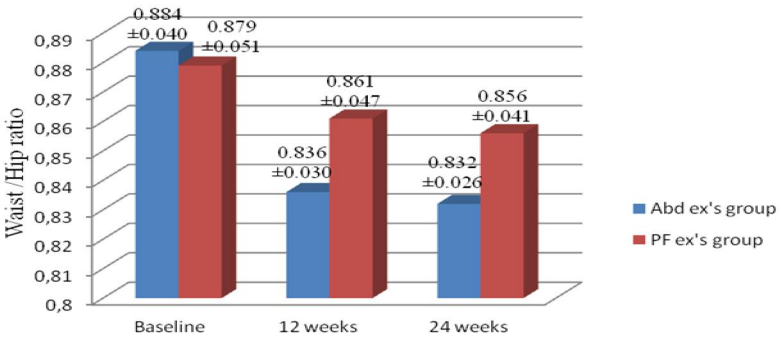

Fig. 3 The mean values of waist /hip ratio (WHR) in both groups

Appendix

Stage	Exercise program	Exercise description
Group (A)	Transversus abdominal muscles exercises	Patients were in crock lying position and were asked to contract statically their abdominal muscles strongly**.** This done for 15 repetitions each one consisted of contraction for 10 sec. followed by relaxation for 20 sec., after 15 repetitions, they were rested for 5 min**.** Then repeat the consequence again for two sets of 15 repetitions. Instructions: the patient's hands over the abdomen to feel its tension. The pelvis and spinal movements were prevented
	Internal obliqus muscles (lateral trunk flexion) exercises	Patients were in crock lying position and were asked to contract statically their abdominal muscles strongly, then try to touch with their tips of the fingers the farthest point of their legs for 15 repetitions each one consisted of contraction for 10 sec. followed by relaxation for 20 sec., after 15 repetitions, they were rested for 5 min. Then repeat the same procedures for the other leg.
Group (B)	pelvic floor muscles exercises	1) **First step for pubovaginalis**:- Patients were asked to contract the anterior fibers of pubococygeus muscle 15 repetitions each one consisted of contraction and squeezing for 10 sec., followed by relaxation for 20 sec., after 15 repetitions, they were rested for 5 min. Perineal palpation was done during the exercise to assure the cranial movement of the perineum.
		2) Second step for puborectalis:- Patients were asked to contract the posterior fibers of pubococygeus muscle 15 repetitions each one consisted of contraction and squeezing for 10 sec., followed by relaxation for 20 sec., after 15 repetitions, they were rested for 5 min. The therapist tips of fingers were around the anus to assure contraction of puborectalis as lifting of the anus up and detect any substitution of gluteus maximums muscles.
		3) Third step for pubo cocygeus as a whole:- Patients were asked to contract the anterior and posterior fibers of pubococygeus muscle 15 repetitions each one consisted of contraction and squeezing for 10 sec., followed by relaxation for 20 sec. Palpation as step 2 was done in addition to verbal instruction to assure raising of the perineum.
Home routine both groups (A&B)		*All patients were given a record for the home routine of exercises. It was done four times per day on the other days of usual exercises in their group, as following: - At early morning before getting from bed from crock lying position. - At afternoon from sitting and standing positions. - At evening from sitting and standing positions. - At night at bed time from crock lying position

4. Discussion

The results of this study suggested that 12 weeks of specific abdominal muscles training showed benefits more than pelvic floor training in improving vaginal pressure and leak point pressure

which indirect positively affect mild SUI in obese patients.

One of our weaknesses is that, BMI assessment was not done post intervention and at the follow up. Furthermore, we relied only on objectives assessments without accounting episodes of leakage or self reported questionnaire.

Regarding the effect of the abdominal muscles exercises on the function of the pelvic floor muscles in mild SUI, to the best of our knowledge, there is no study tested the effect of the abdominal muscles training alone in comparison to the pelvic floor muscles for SUI. But there are many studies supporting the relation between these two groups of muscles. A recent study done by Hung *et al.* [25], who found that 4-months period intervention by retraining diaphragmatic, deep abdominal and pelvic floor muscles (PFM) coordinated function could improve symptoms and quality of life in women with SUI or mixed urinary incontinence (MUI).

WHR had a significant decrease after 12 &24 weeks compared to the baseline in both groups. This can be explained as central adiposity increases the intra-abdominal and bladder pressure and urethral mobility so; weight reduction by changes in dietary intake and physical activity may reduce forces on the bladder and pelvic floor, thus reducing incontinence [26].

In the current study, there were a significant improvements (vaginal pressure, LPP and WHR) obtained after 12 weeks of intervention either abdominal or pelvic floor exercises for obese women with SUI. This is broadly in line with current consensus in muscle physiology that improvements in strength can be observed after 8 weeks of training [27]. Furthermore, even if pelvic floor or abdominal muscles are severely and recently affected as in cases of persistent postnatal stress urinary incontinence, 8 weeks of pelvic floor or pelvic floor plus abdominal training are sufficient to improve pelvic floor strength [23].

Awareness of pelvic floor muscles contraction is individually different and may require the utilization of different techniques. The improvement obtained in Abd. ex's group can be explained as the abdominal muscles act indirectly to activate the pelvic floor muscles and maintain its coordination, support, endurance and strength [15]. In addition, Thompson *et al.* [28] found abdominal muscles were more active than pelvic floor muscle in symptomatic women, and suggested careful monitoring of this phenomenon when teaching pelvic floor muscle contractions. Furthermore, there is more than preliminary evidence that exercises for transversus abdominis and the obliqus internus when recruited lead to activation of the pelvic floor muscle. This was the essential concept behind the regimen of exercises in Abd. ex's group of this study [20,22].

Both abdominal and pelvic floor muscles are affecting each other. This was obvious when healthy subjects co-contracted pelvic floor during low abdominal hollowing in four-point kneeling results in greater increase in transversus abdominis thickness [29]. Furthermore, there was an increase in thickness of the transversus abdominis and internal obliqus muscles during pelvic floor muscle (PFM) contraction showing a co-contraction of the abdominals during PFM contraction both in women with and without SUI [30]. In contrast, EMG biofeedback over abdominal muscles was used for patients suffering from SUI who asked to minimize the abdominal muscles contraction during pelvic floor exercises. It seems that using biofeedback provides no difference between both groups [31]. In addition, Bo [32] concluded that instruction to contract the pelvic floor muscles produces a more effective pelvic floor muscle contraction than instruction to perform a transversus abdominis muscle contraction.

The effectiveness of pelvic floor muscles training for SUI as in PF ex's group can be explained that the pelvic floor contraction enhances closure of the urethra. With this closure, pressure in the urethra is elevated and leakage is avoided. Contraction also helps to maintain urethral position during intra-abdominal pressure increase [33].

The results obtained in PF ex's group are supported by many studies which had shown the effects of pelvic floor exercises as elevation of the bladder neck, increased pelvic floor contraction pressure [34,35], and decrease in volume of leaked urine [36]. Pelvic floor exercises are superior for treating SUI compared with electrical stimulation, biofeedback, vaginal cones, and no treatment [37]. Pelvic floor exercises have a long term benefit for patients after vaginal and cesarean birth [38]. Furthermore, the benefits of pelvic floor exercises can be maintained for up to 5 years even with a reduction in frequency of exercise to as little as one session/week [39].

Finally, we recommend for further studies using another methods of assessment e.g. one pad test. In addition, compare the abdominal versus the pelvic floor exercises in normally weight females with SUI or MUI.

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References

1- Cammu H, Blockeel C, Amy J. (2004). Who will benefit from pelvic floor muscle training

for stress urinary incontinence? Am J Obstet Gynecol.; 191(4): 1152-1157.
2- Symonds I, Baker P, Kean L. (2002). Problem orientated obstetrics and gynecology. London: Arnold- Holdder Heading Group; 257.
3- Doughty D. (2003). Promoting continence: simple strategies with major impact. Ostomy Wound Manage; 49:46-52.
4- McGuire E, Gespedes R. (1996). Proper diagnosis: A must before surgery for stress incontinence. J Endo. Urol.; 10: 201-205.
5- Frawley H, Galea M, Phillips B, Sherburn M, Bo K. (2006). Reliability of pelvic floor muscle strength assessment using different test positions and tools. *Neurourol Urodyn.*; 25:236–242.
6- Luber K. (2004). The Definition, Prevalence, and Risk Factors for Stress Urinary Incontinence. Rev Urol.; 6(Suppl 3): S3–S9.
7- Hannestad S, Rortveit G, Daltveit A, Hunskaar S. (2003). Are smoking and other lifestyle factors associated with female urinary incontinence? The Norwegian Epincont Study. BJOG.; 110**:** 247-254.
8- Hunskaar S. (2008). A systematic review of overweight and obesity as risk factors and targets for clinical intervention for urinary incontinence in women. Neurourol Urodyn.; **27:** 749- 757.
9- Cummings J, Rodning C. (2000). Urinary stress incontinence among obese women: review of pathophysiology therapy. Int Urogynecol J Pelvic Floor Dysfunct.; 11(1):41-44.
10- Bai S, Kang J, Rha K, Lee M, Kim J, Park K . (2002). Relationship of urodynamic parameters and obesity in women with stress urinary incontinence. *J Reprod Med*.; 47:559–563.
11- Yamada B, Govier F. (2006). Does weight loss improve urinary incontinence in overweight and obese women? Nat Clin Pract Urol.; **3**: 16-17.
12- Kegel A. (1948). Progressive resistance exercise in the functional restoration of the perineal muscles. Am J Obstet Gynecol.; 56: 238-249.
13- Kegel A. (1956). Stress incontinence of urine in women: Physiologic treatment. J Int Coll Surg.; 25: 487-499.
14- Markwell S, Sapsford R. (1998). Physiotherapy management of pelvic floor dysfunction. In: Sapsford R, Bullock J, Markwell S (eds). Women's health: A text book for physiotherapist. London: WB Saunders Company,: 383-407.
15- Sapsford R, Hodges W. **(**2001). Contraction of the pelvic floor muscles during abdominal maneuvers. Arch Phys Med Rehabil.; 82: 1081-1088.
16- Madill S, McLean L. (2006). Relationship between abdominal and pelvic floor muscle activation and inter-vaginal pressure during pelvic floor muscle contractions in healthy continent women. Neurourol Urodyn.; 25(7):722-730.
17- Richardson C, Jull G, Hodges P, Hides J. (1999). Therapeutic exercise for spinal segmental stabilization in low back pain. Edinburgh: Churchill Livingstone,:75-84.
18- Power M. **(**1948). Embryological development of the levator ani muscle. Am J Obstet Gynecol.; 55: 367-381.
19- Sapsford R. (2001). The pelvic floor: A clinical model for function and rehabilitation. Physiother.; 87(12): 620-630.
20- Sapsford R, Hodges P, Richardson C, Cooper D, Markwell S, Jull G. (2001). Co-activation of the abdominal and pelvic floor muscles during voluntary exercises. Neurourol Urodyn.; 20:31-42.
21- Sapsford R, Markwell S, Clarke B. (1998). The relationship between urethral pressure and abdominal muscle activity. Aust Continence J.; 4: 102-110.
22- Neumann P, Gill V. (200**2).** Pelvic floor and abdominal muscle interaction: EMG activity and intra-abdominal pressure. Int Urogynecol J Pelvic Floor Dysfunct.; 13(2): 125-32.
23- Dumoulin C, Lemieux M, Gravel D, Morin M. (2004). Physiotherapy for persistent postnatal stress urinary incontinence: a randomized controlled trial. Obstet Gynecol.; 104 (3): 504-510.
24- Singh D. **(**2002). Female mate value at a Glance: Relationship of Waist-to-Hip Ratio to Health, Fedundity and Attraction. Neuro Endocrinol Lett.; 23 (special issue):81-91.
25- Hung H, Hsiao S, Chih S, Lin H, Tsauo J. (2010). An alternative intervention for urinary incontinence: Retraining diaphragmatic, deep abdominal and pelvic floor muscle coordinated function. Man Ther.; 15: 273–279.
26- Subak L, Wing R, West D, Franklin F, Villinghoff E, Creasman J, *et al.* (2009). weight loss to treat urinary incontinence in overweight and obese women. N Engl J Med.; 360: 481-490.
27- DiNubile N. (1991). Strength training. *Clin Sports Med.*; **10**: 33–62.
28- Thompson J, O'Sullivan P, Briffa N, Neumann P. (2006). Altered muscle activation patterns in symptomatic women during pelvic floor muscle contraction and valsalva manouevre. Neurourol Urodyn.; 25(3):268–76.
29- Critchley D. (2002). Instructing pelvic floor contraction facilitates transversus abdominis thickness increase during low abdominal hollowing. Physiother Res Int.; 7 (2): 65-75.
30- Arab A, Chehrehrazi M. (2011). The response of the abdominal muscles to pelvic floor muscle contraction in women with and without stress urinary incontinence using ultrasound imaging. Neurourol Urodyn.;30(1): 117-120.

31- Wong S, Fung Y, Fung M, Tang H. (2001). Biofeedback of pelvic floor muscles in the management of genuine stress incontinence in Chinese women. Physiother.; 87(12): 644-648.
32- Bo K, Sherburn M, Allen T. (2003). Transabdominal ultrasound measurement of pelvic floor muscle activity when activated directly or via a transversus abdominis muscle contraction. Neurourol Urodyn.; 22 (7): 654-658.
33- McIntosh L, Frahm J, Mallet V, Richardson D. (1993). Pelvic floor rehabilitation in the treatment of incontinence. J Reprod Med.; 38(9):662-666.
34- Aukee P, Immonen P, Laaksonen D, Airaksinen O. (2004). The effect of home biofeedback training on stress incontinence. Acta Obstet Et gynaecol Scand.; 83(10): 973-977.
35- Bo K. (2004). Pelvic floor muscle training is effective in the treatment of female stress urinary incontinence, but does it work? Int Uorgynecol J Pelvic Floor Dysfunct.; 15 (2): 76-84.
36- Sampselle M, Miller J, Aims B, Antonakos C. (1998). Effect of pelvic muscle exercises on transient incontinence during pregnancy and after birth. Obstet Gynecol.; 91(3): 406-412.
37- Bø K, Talseth T, Holme I. (1999). Single blind randomized controlled trial of pelvic floor exercises, electrical stimulation, vaginal cones, and no treatment in management of genuine stress incontinence in women. BMJ.; 318:487-493.
38- Karram, M. (1996). Efficacy of non-surgical therapy for urinary incontinence J Reprod Med.; 41(4): 215-219.
39- Bo K, Talseth T. (1996). Long term effect of pelvic floor muscle exercise 5 years after cessation of organized training. Obstet Gynecol.; 87: 261-265.

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To study the effect of adding Fe_2O_3 nanoparticles on the morphology properties and microstructure of cement mortar

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Abstract: In this study, research has been done on the compressive and tensile strength of cement mortar containing Fe_2O_3 nanoparticles in the amounts of 1, 3 and 5% by weight of cement. The results show that the mechanical properties of samples containing 1% and 3% Fe_2O_3 nanoparticles are desirable than the ordinary cement mortar. SEM study about the micro structure of cement mortar containing nanoparticles and ordinary cement mortar showed that Fe_2O_3 nanoparticles fills the pores completely and reduces the large crystals of $Ca(OH)_2$ and the hydrate products are denser and compact. The mechanical properties results showed that by increasing Fe_2O_3 nanoparticles up to 5% reduces the mechanical properties.
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Keywords: Mechanical properties; Fe_2O_3 nanoparticles; cement mortar; SEM, microstructure

1. Introduction

In recent years, much attention is in the application of nanoparticles in civil engineering, because nanoparticles due to its small size possess unique properties such as high specific surface area and high activity. If nano materials are combined with traditional building materials, this may lead to production of building materials with unique properties and be useful for construction industry. On the other hand, building materials based on Portland cement (concrete, cement mortar, hardened cement paste), is one of the most used and important components used in the construction industry. Much research has been done on the partial replacement of cement with supplementary cementing materials such as Pozolon and nanoparticles to improve their mechanical properties. Many researchers have studied the mechanical properties of cement based materials containing nanoparticles.

Researches done shows that adding nano-particles to cement-based materials improves the mechanical properties. Hui Li and et al in 2005 have examined the abrasion resistance of concrete containing TiO_2 and SiO_2 nanoparticles. The experiment results shows that the abrasion resistance of concrete containing nanoparticles has improved and abrasion resistance of concrete containing nano TiO_2 is more than the abrasion resistance of concrete containing nano SiO_2 [2]. Other research results show that adding SiO_2 nanoparticles can improve the microstructure of the cement and result in the increase of freezing resistance with high performance concrete [3]. This is an important property for concrete exposed to frost. Ali Nazeri et al in 2010 proved that addition of TiO_2 and ZnO_2 nanoparticles increases the concrete compressive strength. These particles also reduce the workability of fresh concrete where in this case it is essential in the use of plasticizer in high percentage of nanoparticles [4, 5]. Mohammad Reza Arefi and et al have studied the effect of adding SiO_2 particles with diameters and in different amount to the cement mortar. The research results showed that nanoparticles due to higher specific surface area improve the mechanical properties and water permeability of cement mortar more than the micro-particles [6]. Studies done on the effect of adding nanoparticles to cement mortar shows that the nanoparticles reduces the amount and size of $Ca(OH)_2$ crystals and needle shaped hydrates and takes position as a nucleus in the cement paste and progresses the hydration of cement having high activity. Cause to create a homogenous and denser cement matrix and ultimately leads to the improved mechanical properties of cement mortar [7-9]. Meral Oltulu and et al have investigated separately and combined the effect of adding nanoparticles of Fe_2O_3, Al_2O_3 and SiO_2 to cement mortars containing silica fume. The research results show that adding these nanoparticles separately causes the increase of compressive strength and improved capillary permeability. But, the interaction of nanoparticles as binary and ternary combinations has a negative effect on the physical and mechanical properties of cement mortar [10].

The research done on the addition of Fe_2O_3 nanoparticles to cement-based materials shows that

the cement mortar containing nano-Fe_2O_3 can sense its compressive stresses in elastic and inelastic system. Because nano-Fe_2O_3 can change the electric resistance of cement mortar with the loading applied. This property is useful for the monitoring structural health [11]. The flexural, tensile and compressive strength of concrete is increased and reduces the setting time of concrete [12, 13]. Conflicting results has been shown about the optimized percentage of adding Fe_2O_3 nanoparticles. Lee and et al [7] showed that by adding nanoparticles to cement mortar, samples containing 3% nanoparticles has the highest mechanical properties but, the research results of Ali Nazeri and et al [12, 13] has shown that the best compressive strength is related to the sample containing 1% Fe_2O_3 nanoparticles.

The aim of this study is to find the optimized percentage of adding Fe_2O_3 nanoparticles and to achieve high strength mortar and finding mechanism to improve the mechanical properties of cement mortar.

2. Material and Methods

2.1. Materials and mixture proportions

ASTM C 150 [14] Type II portland cement was used. The superplasticizer was a commercial sulphonated melamine formaldehyde polymer manufactured by vand chemie in Iran with relative density of 1.15. The content was adjusted for each mixture to ensure that no segregation would occur. Also, the distilled water was used for preparing all mixtures. Crushed silica sand was used with apparent density of 3.33 gr/cm^3 and the fineness modulus of 2.6. The sand was graded according to ASTM C33 [15] standard. The largest diameter of these aggregate particles was 4.75mm. The Fe_2O_3 nanoparticles were purchased from Skyspring Nanomaterials Inc. The characteristics of the Fe_2O_3 nanoparticles were shown in Table 1.

Table 1. The characteristics of nanoparticles

Nanoparticle type	Diameter	Specific surface area (m^2/g)	Purity(%)
Fe_2O_3	30 nm	60	99%

The mixture proportions of the ordinary cement mortar and the cement mortar containing Fe_2O_3 nanoparticles were shown in Table 2. The ratio of the water to binder (the cement and Fe_2O_3 nanoparticles) was chosen 0.42. In this study the mixtures were examined with the cement replacement of 1%, 3% and 5% by weight of cement.

Table 2: Mix proportion of samples (kg/m^3)

Sample name	Water	Cement	Sand	Nano Fe_2O_3 particles	*SP
*CO	150	360	1800	-	-
1NF	150	356.4	1800	3.6	3.68
3NF	150	349.2	1800	10.8	4.29
5NF	150	342	1800	18	4.9

*CO: Control- Ordinary cement mortar
*SP: superplasticizer

2-2- Sample preparation

The high homogenous dispersion of nanoparticles strongly depends on stable suspension preparation. Hence Fe_2O_3 nano powder was mixed with the distilled water and stirred for 6-10 hours by rotational speed of 250-300rpm. At first, the suspension of the Fe_2O_3 nanoparticles and the superplasticizer were mixed in the mixer for 30 second, where the cement was added to this mixture simultaneously. Thereafter, the sand, from finest to coarsest, was added gradually to the mixture, and the mixing continued until the complete homogenization of the mixture. Then, the mortar was poured into the standard mold. For tensile test, the briquette specimens with 75×25×25 mm dimension were utilized. The mortar was poured in two layers, both of them compressed by 4 impacts of a steel rod. In order to prepare the specimens of the compressive tests, the mortar was poured into molds to form cubes of size 50×50×50 mm in three layers alternatively, which all layers compressed by 10 impacts of a steel rod. The molded specimens were covered with a plastic layer for 24 hours and then were cured in water at the room temperature up to end of the seventh day. Six specimens were prepared for each test and the average result was reported.

2-3 Test methods

The apparatus made by ELE Company, England was used for performing the mechanical tests. The microstructure of the specimens was studied by the scanning electron microscopy (SEM) Hitachi S-4160. Compressive tests were carried out according to the ASTM C109 [16] and tensile tests were carried out according to the ASTM C190 [17].

3. Results and Discussion

3.1. Microstructure of samples

The microstructure of samples is shown in figure 1. As shown in the figure, it can be seen in the microstructure the ordinary mortar samples of large crystals of $Ca(OH)_2$. The microstructure of cement

mortar is non dense and the voids can be observed. Microstructure of the sample containing 1% nanoparticles is similar to ordinary mortar. In both large crystal $Ca(OH)_2$ is observed with the difference that the voids are reduced and the mortar structure is more denser. With the increase of nanoparticles quantity up to 3%, microstructure has improved completely and achieved better density. As shown in figure 1d, in samples containing 5% nanoparticles because of the agglomeration of nanoparticles voids are formed. These microstructures with the reduction of mechanical properties in these samples are appropriate.

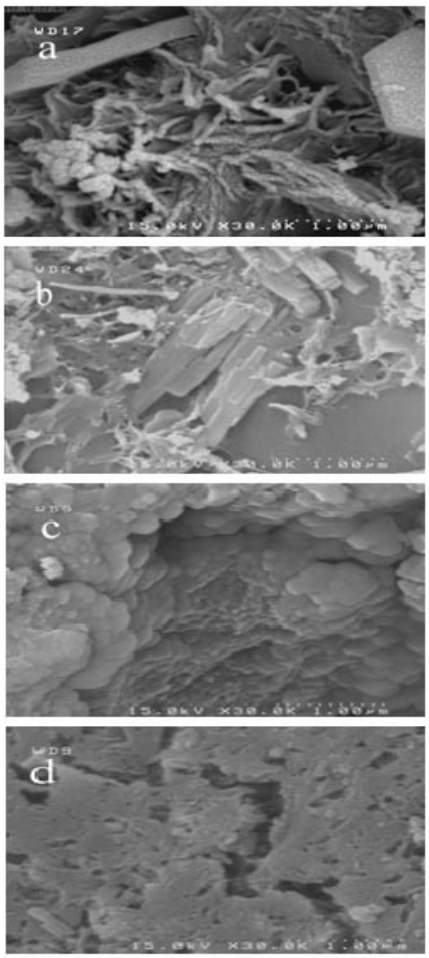

Figure 1. Microstructure of the samples,
a) Sample of CO. b) Sample of 1NF.
c) Sample of 3NF. d) Sample of 5NF

3.2. Mechanical properties

Results of compressive strength, tensile strength after curing for seven days is given in table 3. It can be understood from the table that the sample containing 1 and 3% Fe_2O_3 nanoparticles, the mechanical properties has improved than the ordinary cement mortar. As indicated in figure 1 in a sample containing 1% nanoparticles than ordinary samples the structure of cement mortar is compacted, but still $Ca(OH)_2$ large crystals is observed. With the increasing quantity of nanoparticles up to 3% the $Ca(OH)_2$ large crystals are removed and the microstructure of the mortar is completely compacted. The mechanism of Fe_2O_3 nanoparticles which increases the strength of cement mortar can be described as follows that the addition of Fe_2O_3 nanoparticles reduces the quantity and size of $Ca(OH)_2$ crystals and fills the voids of C-S-H gel structure and ultimately the structure of hydrated products are denser and compact [7]. But, the increased Fe_2O_3 nanoparticles up to 5%, the mechanical properties reduces. This issue is because nanoparticles due to their high surface energy have the tendency towards agglomeration. When nanoparticles are over added to the mortar it is not uniformly distributed in cement mortar and due to agglomeration, weak zone appear in the cement mortar.

This phenomenon can be explained as when the nanoparticles are uniformly distributed in cement mortar each particle has a cubic pattern and distance between the nanoparticles is adjustable. After beginning the cement hydration process the hydrated product are distributed and surrounds the nanoparticles as the nucleus. If the amount and distance between the particles is appropriate, nanoparticles prevents the growth of $Ca(OH)_2$ crystals [2]. The past research of these researchers show that with excessive increase of nanoparticles quantity, the nanoparticles distance decreases and $Ca(OH)_2$ crystals due to limited space cannot grow enough and finally the crystal quantity is reduced [18]. This factor along with the agglomerated nanoparticles causes the mechanical properties of the sample 5NF is lower than the ordinary mortar sample. Thus the effect of the nanoparticles agglomeration and non-desirable influence on the entire structure causes local cracks and ultimately reduces the mechanical properties.

The results show that the addition of Fe_2O_3 nanoparticles, increasing amount of compressive strength is more than tensile strength. The reason for this is the presence of micro cracks in the cement mortar. The impact of these micro cracks on tensile strength is greater than the compressive strength [2, 19].

Table 3: Mechanical properties of samples

Sample name	Compressive strength		Tensile strength	
	(MPa) amount	Percent increased (%)	(MPa) amount	Percent increased (%)
CO	11.96	-	1.51	-
1NF	18.71	56.44	2.03	34.43
3NF	20.81	74	2.25	49
5NF	10.08	-9.7	1.25	-17.22

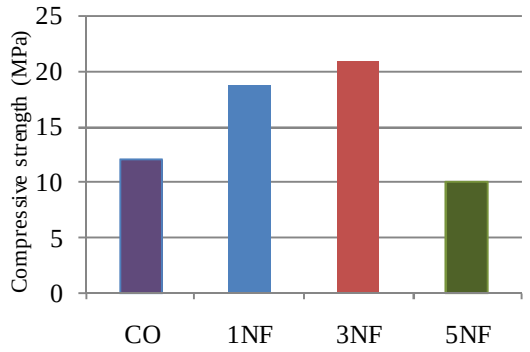

Figure 2. Compressive strength of samples

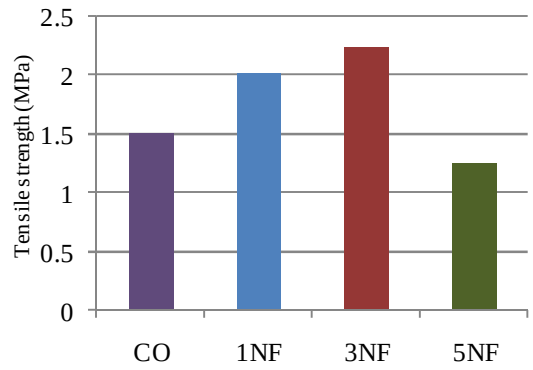

Figure 3. Tensile strength of samples

4. Conclusion

With respect to the experimental results of tensile and compressive strength it is expected that adding of Fe_2O_3 nanoparticles up to 3% by weight of cement can act as a filler for strengthening the micro structure of cement and also reduces the quantity and size of $Ca(OH)_2$ crystals and fill the voids of C-S-H gel structure and finally structure of hydrated product is compacted and denser. With the increase of nanoparticles quantity up to 5% there is decrease in nanoparticles distance and $Ca(OH)_2$ crystal due to limited space cannot grow to appropriate size. This factor along with the agglomerated nanoparticles causes the mechanical properties of the sample 5NF is lower than the ordinary mortar sample.

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References

1. Zhang MH, Li H. Pore structure and chloride permeability of concrete containing nano-particles for pavement. Constr Build Mater 2011; 25: 608–616.
2. Li H, Zhang MH, Ou JP. Abrasion resistance of concrete containing nano-particles for pavement. Wear 2006; 260: 1262–1266.
3. Baomin W, Lijiu W, Lai FC. Freezing Resistance of HPC with Nano-SiO_2. J Wuhan Univ Technol-Mater(Sci. Ed) 2008;23:85-8.
4. Nazari A, Riahi S, Riahi S, Shamekhi SF and Khademno. Assessment of the effects of the cement paste composite in presence TiO_2 nanoparticles. Journal of American Science 2010;6:43-46.
5. Riahi S, Nazari A. Physical, mechanical and thermal properties of concrete in different curing media containing ZnO_2 nanoparticles. Energy and Buildings 2011; 43:1977–1984.
6. Arefi MR, Javaheri MR, Mollaahmadi E, Zare H, Abdollahi Nejand B, Eskandari M. Silica nanoparticle size effect on mechanical properties and microstructure of cement mortar. Journal of American Science, 2011;7:231-238.
7. Li H, Xiao H.G, Yuan J, Ou J. Microstructure of cement mortar with nano-particles. Compos: Part B: Eng 2004; 35: 185–9.
8. Byung-Wan Jo, Chang-Hyun Kim, Ghi-ho Tae, Jong-Bin Park. Characteristics of cement mortar with nano-SiO_2 particles. Construction and Building Materials. 2007;21:1351–1355.
9. Qing Y, Zenan Z, Deyu K, Rongshen C. Influence of nano-SiO_2 addition on properties of hardened cement paste as compared with silica fume. Constr Build Mater 2007;21:539–45.
10. Oltulu M, Sahin R. Single and combined effects nano-SiO_2, nano-Al_2O_3 and nanoFe_2O_3 powders on compressive strength and capillary permeability of cement mortar containing silica fume materials. Science and Engineering A 528 2011: 7012– 7019.
11. Li H, Xiao HG, Ou JP. Study on mechanical and pressure-sensitive properties of cement mortar

with nanophase materials. Cement and Concrete Research 2004;34: 435–438.
12. Nazari A, Riahi S, Riahi S, Shamekhi SF and Khademno. Benefits of Fe_2O_3 nanoparticles in concrete mixing matrix . Journal of American Science. 2010;6:102-106.
13. Nazari A, Riahi S, Riahi S, Shamekhi SF and Khademno. The effects of incorporation Fe_2O_3 nanoparticles on tensile and flexural strength of concrete. Journal of American Science. 2010;6: 90-93.
14. ASTM C150. Standard Specification for Portland Cement. American Society for Testing and Materials;2005.
15. ASTM C33. Standard Specification for Concrete Aggregates. American Society for Testing and Materials;2007.
16. ASTM C109 / C109M. Standard Test Method for Compressive Strength of Hydraulic Cement Mortars (Using 2-in. or [50-mm] Cube Specimens). American Society for Testing and Materials;2008.
17. ASTM C190. Method of Test for Tensile Strength of Hydraulic Cement Mortars. American Society for Testing and Materials;1985.
18. Arefi MR, Mollaahmadi E, Abdollahi Nejand B, Fattah M, High performance self -cleaning cement mortar composite and coats prepared by TiO_2 nanoparticles. Submited in Journal of Materials in Civil Engineering..
19. Li H, Zhang MH, Ou JP. Flexural fatigue performance of concrete containing nano-particles for pavement. Int J Fatigue 2007;29: 1292–1301.

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Synergism between Horse Chestnut (*Aesculus Hippocastanum*) Plant and Two Bacterial Larvicide *(Bacillus thuringiensis Serotype* H-14 And *Bacillus sphaericus* in Controlling the Danque Fever Vector*, Aedes aegypti*

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Abstract: The present study deals with the evaluation of efficiency of two bacterial mosquito larvicides and a plant extract one against *Aedes aegypti* larvae when used in combinations with each other under laboratory conditions, is an integrated method of control . The larvicides formulations used were , *Bacillus thuringiensis* H-14 , *B. sphaericus* 1543-4 and a plant water extract of *Aesculus hippocastanum* date collected after 24 hrs of application under laboratory conditions (27±3˚c) comparing the effect of adding the *B. sph*. Or the horse chestnut to larvae pre-treated for 24 hrs with *B.th*. indicated plant extract addition cause significant increase in larval mortality at the first 24 hrs but with increasing the exposure time no increasing in this mortality percentage. Adding *B. th* . H. 14 to larvae pre-treated with *B. sph*. for 24 hrs is better than using plant extract . No significant difference was found between the efficacy of the extract when it added to larvae pre-treated for 24 hrs with *B.th*. or *B* . *sph*.
[Al-Zahrani, H. A. A and Abuldahab, F. F Synergism between Horse Chestnut (*Aesculus hippocastanum*) Plant and Two Bacterial Larvicide *(Bacillus thuringiensis serotype* H-14 And *Bacillus sphaericus* in Controlling the Danque Fever Vector*, Aedes aegypti*] Life Science Journal, 2011; 8(4):555 -560] (ISSN: 1097-8135). http://www.lifesciencesite.com.

Keywords: Mosquitoes, Biocides, Bacteria, horse chestnut, danque fever larvae, susceptibility, doses, mortality.

1. Introduction

During the past decade, two bacterial mosquito pathogens were used as larvicides in mosquito control programmes, namely, *Bacillus thuringiensis* serotype H-14 and *Bacillus sphaericus* 1543-4. In spite of their relatively high larvicidal activity, yet , the application of the developed commercial formulations under field conditions are still needs further investigations particularly , when these biological control agents are used in integration with other conventional botanical control measures , (Mohan *et al.*, 2001 , Shelton *et al.* , 2002 , Hura *et al.*, 2003 , zhang *et al.* Brar *et al.*, 2006 , Wang *et al.*, 2007. The spore forming gram positive bacterium *B.t.* is an environmentally safe biological insecticide (Rosan *et al.*, 2009). During sporulation *B.T.*produces one or more crystal proteins, which used as biological control agents, Beside using bacteria , botanicals used as safe method also , these botanicals are widely used premium (horse chestnut, *Aesculus hippocastanum* used as an alternative method alone or with bacteria to control the denque fever vector *Aedes aegypti* to evaluate another method (Kumar *et al.*, 2008, Soberon *et al.* , 2009 , Yulin *et al.*, 2010). This plant grow throughout the northern hemisphere although horse chestnut is sometimes called buckeye, it should not be confused with the Ohio or California buckeye trees, which are related but not the same species. For centuries, horse chestnut seeds, leaves, bark and flowers were used in medical requirements.

Horse chestnut seed extract standardized to contain 16 to 20 percent aescin (escin), the active ingredient (national center for complementary and alternative medicine) the nuts contain high concentration of a saponin – class toxins called Aesulin, which is toxic to many animals. In California *Aesculus* kill honey bees. The present work deals with investigating the effect of using mixtures of more than bacterial species as well as mixtures of botanical larvicides and these microbial mosquito larvicides in order to demonstrate the best integrated system that could be used in mosquito control. The aim of the present study is screening of susceptibility of mosquito larvae to botanical and biological (bacterial) between bacterial and botanical mosquito larvicides.

2. Materials and Methods:

1-Test insect

a- ***Aedes aegyptii***

A self perpetuating laboratory colony was raised from a field sampled strain, collected from Jeddah city – Saudi Arabia . The reared colony was maintain under normal day light photoperiods, 27±3˚c and 75±5% RH).Gravid females were allowed to oviposit in plastic cups (10×10cm.) half filled with tap water, the laid eggs were collected daily and separated into bigger enamel pan for larvae hatching. About (25-300) hatched larvae were transferred to round white enamel bowl (35 cm in diameter and 10 cm in height) half filled with tap water . A mixture of bread, dried yeast and dried milk in 2:1:1 ratio, respectively , were ground , sieved and used as larvae food . Pupae were

collected daily by means of a wire mesh and transferred to plastic containers (10×10 cm) half filled with tap water and then introduce inside adult breeding cages . Adult mosquitoes were reared in wooden cages (50×50×50 cm).

Females were fed on blood meals through the introduction of a pigeon into the adult cages. During feeding , cages were covered with black cloth to achieve a more rapid feeding. Plastic cups half filled with tap water were placed inside the cages for egg oviposition a sponge soaked with 10% sugar solution was used for feeding males, from time to time the sponge was replaced by a new one to avoid fungal contamination.

2- Bacterial larvicides

Two mosquito – pathogenic bacteria were used during the present investigation, *Bacillus thuringiensis* serotype H-14 , *Bacillus sphaericus* strain 1593-4 , both bacterial species are belonging to family , Bacillaceae . a commercial formulation in the form of a flowable concentrate under the name of " TEKNAR" , SAN -402 based on *B.th*. H -14 , produced by Sandoz Co. and a *B. sph*. strain 1593-4 in the form of a wetable power produced by SOLVAY- Belgium . Botanical insecticide used was the horse chestnut (premium) , *Aesculus hippocastanum* , prepared by grinding its parts .

3- Bioassays

a)Preparation of the bacterial concentrations :-

For *B.th*. serotype H-14, a suspension was prepared by adding 1 ml of commercial formulation to 1000 ml distilled water, then prepare required serial concentrations .

In Case of *B.sph* 1593-4, a suspension was prepared by adding 0.25 ml distilled water and serial dilutions were carried out. All bacterial suspensions were vigorously shacked prior to each use and freshly prepared and expressed in ports per million (ppm) in order to calculate the LC_{50}. Plant extract concentrations were prepared by dissolving dry grinding parts in ethyl alcohol and serial concentrations were carried out .

b)Combined effect of bacterial and botanical extract :-

The combined action of the botanical extract and each bacterial larvicides, as well as the effect of the two bacterial preparation together were tested the same technique previously mentioned for the determination of the median lethal levels was followed, instead of using one larvicide , one ml from the concentrations which produced 50% mortality of each larvicide was added to plastic cup containing 98 ml distilled water simultaneously susceptibility experiments of larvae that were pre-treated with the bacteria or the chemical larvicide for 24 hrs and then treated with the order larvicides were carried out in six different combinations in table (1) as follows :-

Table (1):Different combinations of larvicides

Pre- treatment	Additional treatment after 24 hrs
1-*B.t* H-14	*B. sph*
2-*B.t* . H-14	*A. hippocastunum*
3- *B . sph*	*B . t . H-14*
4- B . sph	*A . hippo.*
5- A. hippo.	*B . to H-14*
6- A. hippo.	*B . sph*

Notes

1)*B. t* H – 14 *(Bacillus thuringiensis H-14).*
2)B. sph (Bacillus sphaericus) .
3)A. hippo . (Aesculus hippocastanum)
.

C) Mortality reading:

Mortality date were recorded 24, 48 , 72 hrs after treatment . Percentage of mortalities was corrected by Abbott's formula when the mortality among control experiment exceeds 5%.

d) Statistical analysis of data :-

Percentage of mortality was converted into probits, which were then plotted against the concentrations. Median lethal concentration (LC_{50}) was determined.

T-test was used to evaluate the effect of all deferent larvicides either when they were used separately, in mixtures of two preparations or when one preparation was firstly applied when followed by another different preparation 24 hours later.

3. Results

3. 1- Comparative susceptibility of *Aedes aegyptii* Larvae to the larvicides mixtures.

The larvae mortality resulted from treating the 3rd instar larvae of *Aedes aegypti* with the following mixture of:

***a-* (Bacterial + botanical) larvicides .**
a-1(Bacillus thuringiensis + A. hippo.) mixture .
a-2(Bacillus sphaericus + A. hippo.) mixture .
b. Bacterial mixture
(B- thuringiensis + B. sphaericus)

Mixture were represented in table (2) as follows :-

The data in table (2) indicates that , there is a significant increase in larvae mortality occurred from the use of larvicides mixture(pl0.05) . Comparison between the combined effect of the mixture of (*B.t + A.hippo*.) and the mixture of (*B. sph. + A.hippo*.) indicate that there is no significance

difference between the mortality percent occur to 3 rd instar *A. aegyptii* larvae at 24 and 48 hr exposure tim , but at 72 hr exposure period there is a significance difference show that the (*B.sph* . + *A.hippo*.) mixture is effective ($p<0.05$). Also comparison between the combined effect of the bacterial mixture (*B.t* .+ *B.sph*.)and *(B.t* . +*A. hippo*). Mixture indicate that the first mixture have a significant effect that the second mixture ($p<0.05$), while , the bacterial mixture (*B.t* + *B. sph*.) have highly significant effect than (*B. sph* + *A. hippo*.) mixture ($p<0.05$) .

Table (2) : Percentage mortality of the early 3rd instar Larvae of *Aedes aegyptii* treated with mixtures of (*B.t* + *A.hippo.) , (B.sph.+A.hippo) or (B.t + B. sph.).*

Average of corrected mortality percent			
Combined mixture of LC50s of the tested larvicides	**Exposure time**		
	24 hrs	**48 hrs**	**72 hrs**
(*B. t* H-14 + *A. hippo*) 0.037 ppm + 0.013 ppm	**X ± SE**	**X ± SE**	**X ± SE**
	70.80 ± 0.28	**81.04 ± 0.56**	**88.32 ± 0.44**
(*B. sph.* + *A. hippo* 2.6 ppm + 0.013 ppm)	**69.50 ± 0.83**	**85.84 ± 0.97**	**94.30 ± 0.78**
(*B.t* H-14+*B. sph*) 0.037 ppm + 2.6 ppm	**78.82 ± 0.08**	**96.91 ± 0.34**	**100**

2. Effect of using the bacterial and botanical Larvicides on *Aedes aegyptii* Larvae in sequence:

The susceptibility of the 3rd instar larvae of *A. aegyptii* to the toxic effect of the entomopathogenic bacteria , *Bt*. H-14 or *B. sph* when pre-treating the larvae with it for 24 hrs and then the other bacteria or *A. hippo*. was added, were evaluated through carring out a series of laboratory experiments. The results obtained in table (3) indicated that, adding *B. sph*. To larvae pre-treated with *Bt*. for 24 hrs cause significant mortality than if *B. sph*. was used alone also , adding *A. hippo*. Resulted in a significant larval mortality than if botanical was used alone at first 24 hrs , but with increasing exposure time no significant mortality occur when adding *B. sph.*

Treating the 3rd instar of *A.aegyptii* larvae with *B. sph*. For 24 hrs with LC_{50} (2.6 PPm) then *Bt* H-14 or *A.hippo*. larvicides was added in order to evaluate its effectiveness were represented in table (4) and indicating that , pretreating larvae with *B. sph*. For 24 hrs then *Bt*. was added cause a significant increase in larval mortality than if *Bt* was used alone.

Table(3) : Percentage mortality of the early 3rd instar larvae of *Aedes aegyptii* pre-treated with *Bt*. H-14 , LC_{50} (0.037 PPm)for 24 hrs to *B. sph* , LC_{50}(2.6 PPm) or *A. hippo.* , LC_{50}(0.013 PPm).

Post-Exposure time	***B. sph.***		***A. hippo***		**Average of corrected mortality percent.**	
	LC_{50} ppm	**LC_{90} ppm**	**LC_{50} ppm**	**LC_{90} ppm**	**Larvae pre-treated with *B. t.* for the first 24 hr with LC_{50} conc. = 0.037 ppm**	
24 hrs	**2.6**	**8**	**0.013**	**0.027**	**Larvae post-mixed treatment with B. sph**	**Larvae post-mixed treatment with *A.hippo*..**
					X ± SE	**X ± SE**
					77.30 ± 0.05	**88.18 ± 0.39**
46 hrs	**1.18**	**2.7**	**0.011**	**0.022**	**99.99 ± 0.01**	**96.12 ± 0.98**

Table (4): Percentage mortality of the early 3rd istar larvae of *Aedes aegyptii* pre-treated with *B. sph*. 1593-4 , LC_{50}(2.6 ppm) for 24 hrs to *B.t* ,LC_{50}(0.037ppm) or *A. hippo*. LC_{50}(0.013ppm).

Post-Exposure time	**B. t. i.**		***A. hippo***		**Average of corrected mortality percent.**	
	LC_{50} PPm	**LC_{90} PPm**	**LC_{50} PPm**	**LC_{90} PPm**	**Larvae pre-treated with *B. sph.* for the first 24 hr with LC_{50} conc. = 2.6 PPm**	
24 hrs	**0.037**	**0.15**	**0.013**	**0.027**	**Larvae post-mixed treatment with *B. t.* H-14**	**Larvae post-mixed treatment with *A. hippo***
					X ± SE	**X ± SE**
					90.02 ± 0.35	**85.95 ± 0.21**
46 hrs	**0.022**	**0.1**	**0.011**	**0.022**	**100**	**99.01 ± 0.30**

3. Effect of pre-treating *Aedes aegyptii* larvae with *A. hippo*. for 24 hrs then bacterial larvicides was added .

Finding the potential efficacy of *A. hippo*. Larvicide when the tested larvae were pretreated with it for 24 hrs with the LC_{50} (0.013PPm) and then *B. t*. or *B. sph*. was added , were done through carrying out a series of laboratory experiments and the results were tabulated in table (5) . The results indicated that , pretreatment with *A. hippo.* Then adding *B.t*. or *B.sph* cause a significant increase in larval mortality.

Table (5) : Percentage mortality of early 3rd isstar larvae of *A. aegyptii* pre-treated with *A.hippo* LC_{50} (0.013ppm) for 24 hrs to B. t. H-14 , LC_{50} (0.037ppm)or *B.sph*. 1593-4 , LC_{50}(2.6ppm)

Post-Exposure time	*B. t* H-14		*B. sph.*		Average of corrected mortality percent.	
	LC_{50} ppm	LC_{90} ppm	LC_{50} ppm	LC_{90} ppm	Larvae pre-treated with *B. t.* for the first 24 hr with LC_{50} conc. = 0.037 PPm	
					Larvae post-mixed treatment with B. t H-14.	Larvae post-mixed treatment with *B.sph.*
					X ± SE	X ± SE
24 hr	0.037	0.15	2.6	8	92.11 ± 0.10	88.99 ± 0.31
46 hr	0.022	0.1	1.18	2.7	97.26 ± 0.04	98.87 ± 0.40

4- Comparative susceptibility of *Aedes aegyptii* larvicide mixture when used in sequence.

The results of pre-treating the *A. aegyptii* larvae for 24 hrs with bacterial larvicides and then the other botanical or bacterial larvicides was added and vice-versa was represented in table (6) as follows :-

No significant differences occurred to larval mortality rates when the tested larvae was pretreated with the two bacterial biocides and then *A. hippo* was added , ($p<0.05$). Mortality occurred as a result of pretreating larvae with *B.sph*. Followed by *B.t*. was found to be significant ($p<0.05$). while no significant difference was obtained as treatment with *A. hippo*. And followed by *B. sph*. or *B. t*. ($p<0.05$).

Table (6): Comparative susceptibility of early 3rd *Aedes aegyptii* larvae pre-treated with *A. hippo., B.t.* H-14 or *B.sph*. 1593-4 for 24 hrs . then the other larvicides were added .

Larvae pre-treated for 24 hrs with	The added larvicide	Averages of corrected mortality percent	
		Exposure time	
		24 hrs	48 hrs
B. thuringiensis Serotype H-14	*B. Sphaericus* *A.hippo*.	X ± SE	X ± SE
		77.30 ± 0.05	99.99 ± 0.01
		88.95 ± 0.39	96.12 ± 0.98
B. Sphaericus 1593-4	*Bti* H-14	90.02 ± 0.35	100
	A.hippo	85.95 ± 0.21	99.01 ± 0.40
A. hippo	Bti H-14	92.11 ± 0.10	97.26 ± 0.04
	B. Sphaericus	88.99 ± 0.31	98.87 ± 0.40

4. Discussion and conclusion

When toxins from B.t. subsp. *Israelensis* or B. *sphaericus* were combined with a botanical extract of *A. hippocastanum* synergestic interactions increased toxicity and expanded the effect range of the mixtures. where as synergy is well documented among the native endotoxins of *B. t.* subsp. *Israelensis* , it is evident from this and earlier studies with cytolytic toxins that synergy is not limited to native toxins combinations but can occur between toxins from unrelated bacterial strains , i. e. between toxins of *B. spharaericus* , *B. t i*. or botanical extract.

Mixtures of *B. sph.* , *B. t. i.* were very active against the normally insensitive mosquito species *Ae. Aegypti* . Toxicity values also were generally not significantly different from those of *A. hippocastanum* those same mixtures showed improved activity against susceptible *A. aegytii* , demonstrating that activity improvement can be achieved toward normally sensitive mosquito as well . That activity is due in part to the inherent toxicity of B. t. i. toxins. however, additional

activity must be attributed to the synergism of *B. sphaericus* (Wirth *et al*., 2000 a, b, c) and synergy involving bacterial toxins . Whether that activity well translate into improved field toxicity remains to be seen .

The broad spectrum of synergy that is now apparent suggests that complex interaction occur among most toxins in *B. t. i. , B. sphaericus* and are probably responsible for much of the increased toxicity (Bourgouin *et al*., 1990, Doncel *et al.* , 1997, Thiery *et al*., 1998, Servant *et al*., 1999 , Li *et al*., 2000 , Sun *et al*., 2001 , Zahiri *et al*., 2002 .

These interaction should provide some level of protection against insecticide resistance because they involve toxins that target different receptors in mosquito midgut (Silva – Filha *et al*., 1999) interact synergistically , traits that naturally occur in *B. t. i.* and B.t. jegathesan (Selena *etal*.,1995) and are believed to retard the evolution of resistance to those two bacterial species (Geoeghiou and Wirth, 1997, Wirth *et al*., 2004) . Furthermore , a mixture of *B. t.* and *B. sph*. Was experimentally demonstrated to delay *B. sph*. Resistance (Zahiri and Mulla, 2003 , Abozinadah *et al*., 2011 and Abuldahab *et al*., 2011 .

B. sphaericus , because of its high activity in polluted water and long residual activity , has an important role in mosquito larval control that is at risk because of its limited host range and propensity to select resistance the risk for resistance potentially reduced by combinating *B. sphaericus* with toxins from B. t. i and active groups of botanical extract (Yulin *et al*., 2011). Botanicals also used as biological control agents due to its ability to reduce mosquito resistance and as a safe method against the target insect (Abuldahab and Younes,1999; Gusmao *et al*. , 2002 Cavados *et al.* 2004 , Amusan *et al.* 2005 , Abdel Halem, 2006 and Nalhan, 2007). Based on the experimental results of bioassaying each of the three tested larvicides individually or in combinations , as well as on studying their pathological action, it may be assumed that it is preferable to use these biological larvicides in integration with the chemical one in mosquito control programs particularly in larval control , instead of using each of them alone .

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Reference

Abdel Halim , A. S. (2006):Efficacy of trigonella foenum graecum on third stage larvae and adult fecundity of *Anopheles pharoensis* . J. Egyp. Soc. Parasitol. , 36 (2):397-404.

Abozinadh, W. Y., Abuldabab, F.F. and AL- Haiqi (2011) : Study of using the bacterium *Bacillus thuringiensis israelansis* in microbial controloy Musca domestica Vicina (Diptera:Muscidae) J. Entom.α Nemato., 3(4):58-670.

Abuldahab , F. F. and Younes , M. W. F. (1999): Effect of three different plant extracts as a tool in pest control of *Musca domestica* (Diptera:Muscidae). Bull. Fac . Sci . Zagazig Univ. , (2):73-88.

Abulolahab, F. F., Abozinaolab., N.Y and AL_Haiqi, N.S. (2011):Impact of *Bacillup thuringiensis* B_exotoxin to some biochemical aspects of *M. domestica* (Diptera: Muscidae). J. Bacter. Ros., 3(5):158-1670.

Amusan , A.A., Idowu , A.B. and Arowoo , F. S. (2005) : Comparative toxicity effect of bush tea leaves (*Hyptis suaveolens*) and orange peel (*Citrus sinensis*) oil extract on larvae of the yellow fever mosquito *Aedes aegypti* .Tanzan Health Res. Bull . 7(3):174-8.

.

Bourgouin , C., A. Delecluse, F. De La Torre, and J. Szulmajster (1990):Transfer of the toxin protein genes of Bacillus *sphaericus* into *Bacillus thuringiensis* subsp. *israellensis* and their expression. Appl. Environ. Microbiol., 56:340-344.

Cavados , C. F. , Majerowicz , S., Chaves , J. Q. : Araujo-coutinho, C. J. and Rabinovitch , L. (2004): Histopathological and ultrastructural effects of delta- endo – toxins of *Bacillus thuringiensis* serovar *israelensis* in mid gut of Simulium pertinax larae (Diptera , Simuliidae) mem. Inst. Oswaldo . Cruz. , 99 (5) : 493-8.

Georghiou, G. P., and M. C. wirth. (1997): Influence of single versus multiple toxins of *Bacillus thuringiensis* subsp. *israelensis* on development of resistance in the mosquito *Culex quinquefasciatus*. Appl. Environ. Microbiol., 63:1095-1101.

Georghiou, G.P., and M.C. Wirth. 1007. Influence of single versus multiple toxins of *Bactllus thuringiensis* subsp. *Israelensis* on development of resistance in the mosquito Culex quinquefasciatus. Appl. Environ. Microbiol., 63:1095-1101.

Gusmao , D. S. , Pascoa , V., Mathias, L., CurCino Vieira, I. J. (2002): Derris Lonchocarpus urucu , extract modifies the peritrophic matrix structure of *Aedes aegypti* (Diptera : Culicidae) Mem . Inst. Oswaldo . Cruz. 97(3) :371-5.

Li , T., F. sun , Z. Yuan , Y.Zhang, J. Yu, and Y. pang .(2000) Coexpression of cytiAa of *Bacillus*

thuringiensis subsp. *israelensis* with *Bacillus sphaericus* binary toxin gene in a crystalliferous strain of *B. thuringiensis* . Curr. Microboil., 40:322-326.

Abuldahab, F. F. , Abozinadah, N.Y and Al- Haiqi , N. S. (2011): Impact of *Bacillus thuringiensis* B-exotoxin to some Biochemical aspects of M. domestica (Diptera:Muscidae. J. Bacter. Res., 3 (5) :158-167.

Li,T.,F.Sun, Z.Yuan, Y.Zhang, J. Yu, and Y.Pang. (2000): Coexpression of cytlAa of *Bacillus thuringiensis* subsp. *israelensis* with *Bacillus sphaericus* blanary toxin gene in acrystalliferous strain of B. thuringiensis. Curr. Microbiol., 40:322-326.

Nathan , S. S. (2007): the use of *Eucalyptus tereticonis*m .(Myrtaceae) oil (leaf extract) as anatural larvicided agent against the malaria vector *Aropheles stephensi* Liston , (Diptera: Culicidae). Bioesour Techal ., 98(9): 1856-60.

Poncet , S., C. Bernard , E., Dervyn, L. Cayley, A. klier , and G. Rapoport .(1997): Improvement of *Bacillus sphaericus* toxicity against dipteran larvae by integration , via homologous recombination, of the CryIIA toxin gene from *Bacillus thuringiensis* subsp. *israelensis* . Appl. Environ. Microbial., 63:4413-4420.

Selena, P.,H. I. Lee, and M. M. Lecadet. (1995): A new serovar of *Bacillus thuringiensis* possessing 28a28c flagellar antigenic structure *: Bacillus thuringiensis* serovar jegathesan, selectively toxic against mosquito larvae . J. Amer . Mosq. Control Assoc ., 11:471-473.

Servant , P., M. L Rosso , S . Hamon, S. poncet, A. Delecluse, and G. Rapoport (1999): Production of CryIIA and CryIIA Ba toxins in *Bacillus sphaericus* confers toxicity towards *Aedes aegypti* and resistant *Culex* populations. Appl. Environ. Microbiol., 65:3021-3026.

Silva- Filha , M., H., C. Nielsen – LeRoux , and J. F. Charles . (1999). Identification of the receptor for *Bacillus sphaericus* crystal toxin in the brush border membrance of the mosquito *Culex pipiens* (Diptera:Culicidae).Insect Biochem Mol. Biol., 29:711-721.

Sun, F.,Z. Yuan, T. Li, Y. Zhang , J. Yu , and Y. Pang .(2001). Reduction of resistance of *Culex pipiens* larvae to the binary toxin from *Bacillus sphaericus* by coexpression of cry4Ba from *Bacillus thuringiensis* subsp. *israelensis* with binary toxin gene. World J. Microbiol. Biotechnol., 17:385-389.

Thiery, I., S.Hamon,A.Delecluse, and S. Orduz . (1998). The introduction into *Bacillus sphaericus* of the *Bacillus thuringiensis* subsp. Medellin cytlAbl gene results in higher susceptibility of resistant mosquito larva populations to *B. sphaericus*. Appl.Environ. Microbiol., 64:3910-3916.

Wirth, M. C., C. P. Georghiou, and G.H. Abro. (2000b). Laboratory selection for resistance to *Bacillus sphaericus* in Culex quinquefasciatus (Diptera: Culicidae) from California. J. Med. Entomol.,37:534-450.

Wirth, M. C., W. E. Walton, and B. A. Federici. (2000c). CytlA from *Bacillus thuringiensis* restores toxicity of *Bacillus sphaericus* against resistant Culex quinquefascia-tus (Diptera: culicidae) .J. Med. Entomol., 37: 401-407.

Wirth,M.C., B. A. Federici, and W. E. Walton (2000a). CytlA from *Bacillus thuringiensis* synergizes activity of *Bacillus sphaericus* against *Aedes aegypti*. Appl. Environ. Microbiol., 66:1093-1097.

Yulin Gao , Juan Luis Jurat – Fuentes , Brenda oppert , Jeffrey A. Fabrick , Chenxi Liu , Jianhua Gao and Zhongren Lei (2011): Increased Toxicity of *Bacillus thuringiensis* Cry3Aa against *Crioceris quatuorde cimpunctata* , *Phaedon brassicae* and *Colaphellus bowringi* by a *Tenebrio molitor* cadherin fragment . (Wiley online library . com) DOI 10.1002/ Ps . 2144.

Zahiri, N.S.,T. Su, and M.S.Mulla. (2002). Strategies for the management of resistance in mosquitoes to the microbial control agent *Bacillus sphaericus*. J. Med. Entomol., 39: 513-520.

Zahiri,N.S., and M. S. Mulla. (2003). Susceptibility profile of Culex quinquefaciatus (Diptera: Culicidae) to *Bacillus sphaericus* on selection with rotation and mixture of *B. sphaericus* and *B. thuringiensis israelensis*. J. Med. Entomol., 40: 672-677.

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Biochemical and Ultra Structure Studies of the Antioxidant Effect of Aqueous Extract of Hibiscus Sabdariffa on the Nephrotoxicity Induced by Organophosphorous Pesticide (Malathion) on the Adult Albino Rats

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Abstract: Organophosphorous (OP) pesticide is applied to numerous crops, including wheat and corn. Residual amounts of organophosphorous pesticides have been detected in soil, vegetables, grains and other food products. Several mechanisms of the OP toxicity have been proposed, including the induction of cellular proliferation, oxidative stress and immune-toxicity. Roselle (ROS, *Hibiscus sabdariffa* L., family Malvaceae) is an annual shrub commonly used to make jellies, jams and beverages. Many biological activities have been recorded for ROS, such as anti-atherosclerosis, anti-carcinogenic, hepato-protective and anti-oxidative properties**.** ***The Aim:*** this study was set to evaluate the possible protective effect of Roselle on nephro-toxicity induced by sub-lethal dose of Malathion in rats. ***Material and Methods:*** 24 adult male albino rats were used and divided into four groups of 6 rats/each. Group I: animals were given corn oil at a dose of 0.2 ml per animal via gavage once a day for one month and served as a control. Group II: animals received only aqueous extracts of Roselle at a daily dose of (500 mg /kg b. wt./day). Group III: animals were given Malathion at a sub lethal dose of 27mg/kg b. wt./day. Groups IV: animals were given both of aqueous extracts of Roselle as the same dose of group II three hours before the administration of Malathion. At the end of the experimental period the kidney function and markers of oxidative stress were investigated. Moreover, histopathological examination of the renal tissue was carried by light and electron microscopes. ***The results*** of the present study showed that treatment with Malathion alone caused increase in the kidney weight ($P<0.001$), cellular degeneration, necrosis of the renal tissues and increase in the serum urea and creatinine ($P<0.001$ for both). However, administration of aqueous extract of Roselle prior to Malathion resulted in a significant alleviation of the kidney injuries evidenced by a decrease of the kidney weights when compared to the Malathion-treated ($P<0.001$) and biochemical indices; urea and creatinine ($P<0.001$; $P>0.05$, respect.) for both when compared to the Malathion-treated and control groups, respectively. Furthermore, there was significant improvement of the histological picture toward the normal among the Malathion+ROS-treated group. All these effects may be due to the antioxidant effect of the Roselle as treatment with the extract of *Roselle* significantly elevated ($P<0.001$) the decreased CAT activity observed with Malathion treated rats. Moreover, treatment with the extract of *Roselle* significantly elevated the SOD levels when compared to the Malathion-treated animals ($P<0.001$). Furthermore, the GSH level reduced significantly ($P<0.001$) along with increased in MDA concentration ($P<0.001$) in the Malathion treated group as compared to the control group. However on treatment with *Roselle* extract, the GSH level was found to be enhanced significantly ($P<0.001$) and the MDA contents were reduced ($P<0.001$) when compared to the Malathion treated group. ***Conclusion***, the results of the current study showed that the aqueous extracts from *Hibiscus sabdariffa* possess a potent protective effect against the oxidative stress induced by sub lethal dose of Malathion on the rat kidney.

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Introduction

In agricultural settings, Malathion an organophosphorous (OP) pesticide is applied to numerous crops, including wheat and corn. It is also used for home and garden applications, mosquito control, Mediterran-ean fruit fly eradication and as a topical treatment for head lice (***Gervais et al., 2009***). Malathion is also an ingredient in shampoos regulated by the United States Food and Drug Administration (FDA) to control head lice (***Frankowski, 2004***).

The exposure to Malathion became an ongoing basis as there are residual amounts of organophosphorous pesticides have been detected in soil, vegetables, grains and other food products (***Iarc, 1983).***

Several mechanisms of the OP toxicity have been proposed, including the induction of cellular proliferation (***Cabello et al., 2001***), oxidative stress (***John et al., 2001 and Abdollahi et al., 2004***) and immunotoxicity (***Galloway and Handy, 2003***). OP

pesticides are known to induce toxicity in mammals by inhibiting acetyl cholinesterase which leads to accumulation of acetylcholine and the subsequent activation of cholinergic muscarinic and nicotinic receptors **(*Hazarika et al., 2003*).** Acetylcholinesterase enzyme (AChE) is found in mammals, amphibians, fish, reptiles, and birds (***Massoulie and Bon, 1982*).** In these organisms, the binding of AChE with Malathion allows the accumulation of ACh at the nerve junction. This accumulation of ACh leads to over stimulation of glandular cells, autonomic ganglia, the central nervous system, and both smooth and skeletal muscles **(*Reigart and Roberts, 1999*).**

Researchers administered Malathion orally reported that more than 90% of the dose was excreted in urine within 24 hours. The remaining Malathion was found in feces, blood, intestines, liver, and kidneys. Also, four hours after oral administration, 75% of the Malathion was still in the stomach, while 8% was in the small intestines, and 7% in the saliva (***Zeid et al., 1993*).** Researchers noted suppression of thyroid secretory function in young adult rats that were fed 0.06 mg/rat/day of Malathion for 21 days. They also noticed an increase in thyroid stimulating hormone (TSH), suggesting that the pituitary gland was compensating to restore normal levels of thyroid hormones **(*Akhtar et al., 1996*).**

Much attention has been focused on the protective effects of anti-oxidants and naturally occurring substances against oxidative stress damage. Roselle is a nature's generosity which providing mankind with cheap and natural bioactive materials. Thus the exploitation of this natural gift is necessary to overcome the unwanted side effects of some essentially used medications. Roselle (ROS, *Hibiscus sabdariffa* L., family Malvaceae) is an annual shrub commonly used to make jellies, jams and beverages. In folk medicine, ROS has commonly been used for its antihypertension properties (***Herrera-Arellano et al., 2004***). The anthocyanin pigments that confer ROS's color make it a valuable food product **(*Tsai and Huang, 2004*).** Many biological activities, such as anti-atherosclerosis, anticarcinogenic **(*Tsai et al., 2002*)**, hepatoprotective (***Amin and Hamza, 2005*)** and anti-oxidative properties **(*Prenesti et al., 2007*)**, have been reported in Ros and its anthocyanin.

Botanically, it is described as thick red fleshly, and cup shaped calyxes plant. The calyxes are rich in phenolic compounds with marked physiological activities (***Rosendiz-Lopez et al., 1998***). Hibiscus flowers contains gossypectin, glucoside, bibiscin, hibiscus anthocyann and protocatechuic acid, which may have diuretic and choleretic effects, decreasing the viscosity of the blood, reducing blood pressure, and stimulating intestinal peristalsis (***Alli and Salih, 1991***). Hibiscus protocatechuic acid (PCA) was shown to significantly decrease the leakage of lactate dehydrogenase (LDH) and alanine transaminase (ALT) and the formation of malondialdhyde (MDA) induced by tert-butylhydroperoxide (t-BHP) in rat primary hepatocytes (***Tseng et al., 1997a***).

The anti-inflammatory activity of this plant has been traced partially to its phenolic acid composition. Another potent antioxidant and cytoprotective agent characterized in *H. sabdariffa* is betaine, which can also account for its antioxidant property **(*Rossi et al., 2003*).**

Hibiscus sabdariffa L. has been reported to be antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, refrigerant, sedative, stomachic and tonic (***Morton, 1998*).**

Numerous *in vitro* experiments have evaluated the effects of hibiscus flower or anthocyanin extracts against various cancer cell lines. Proposed mechanisms of action focus on antioxidant activity and the ability to induce apoptosis (***Lin et al., 2007; Lo et al., 2007; Olvera-García et al., 2007*).** Studies in rats have evaluated effects against liver, oral, colon, bladder, and stomach cancers **(*Ali et al., 2005*).**

Nowadays the exposure to residual of OP compounds is an imperative process because its wide use in agriculture and this chronic exposure may be the cause of wide spread of nephro-toxicity. Based on these, this study was designated to evaluate the possible protective effect of Roselle on nephro-toxicity induced by sub-lethal dose of Malathion in rats in trial to found save natural, potent and effective protective procedure.

2. Material and Methods:

Animals:

Twenty four adult male albino rats (weight 185-200 g) were housed at the animal house in the Faculty of Medicine for Girls Al-Azhar – University at 21–22°C in a 12 hr/12 hr light/dark cycle, fed standard rat chow, and given free access to water. Rats accommodated to the laboratory conditions for 2 weeks before starting the experiment.

Experimental design:

Rats were divided into four groups of 6 rats/each:

Group I: animals were given corn oil at a dose of 0.2 ml per animal via gavages once a day for one month and served as a control group.

Group II: animals received only aqueous extracts of Roselle at a daily dose of (500mg/kg b. wt./day) for one month according to **Amr et al. (2008).**

Group III: animals were given Malathion at a dose of 27mg/kg b. wt./day in corn oil via gavages for one month .This dose equal to 1/50 of the LD_{50} for an oral dose according to **Gallo and Lawryk (1991) and Kamrin (1997).**

Groups IV: animals were given both of aqueous extracts of Roselle as the same dose of group II (500 mg/kg/day) three hours before the administration of Malathion as the same dose of group III (27mg/ kg b. wt. / day) for one month.

At the end of the experimental period (one month) and after overnight fasting, at 8:00 a.m, blood samples were obtained from sinus orbitus vein of each rat after ether inhalation (***Yang et al., 2006***). The blood samples were allowed to clot at room temperature before centrifuging at approximately 3000 rpm for 15 minutes. The serum was stored at -20° C until assayed for the biochemical parameters. Then, all studied animals were sacrificed; the two kidneys of each rat were excised and weighed then prepared for estimation of the markers for oxidative stress and histopathological study. One kidney for estimation of oxidative stress markers and light microscope and the other one prepared for transmission electron microscopical study.

Preparation of renal homogenate:

The kidneys were removed and dissected free from the surrounding fat and connective tissue. Each kidney was longitudinally sectioned, and renal cortex was separated and kept at −8°C. Subsequently, renal cortex was homogenized in cold potassium phosphate buffer (0.05 M, pH 7.4). The renal cortical homogenates were centrifuged at 5000 rpm for 10 min at 4°C. The resulting supernatant was used for the determination of malondialdehyde (MDA) content, reduced glutathione (GSH) levels and antioxidant enzyme levels such as superoxide dismutase (SOD), and catalase (CAT), using colorimetric assay**.**

Histopathological preparation for light microscopic examination:

Three pieces of kidneys tissues from each group were fixed in Bouin's solution for 48 hrs. Later, they were dehydrated in graded levels of ethanol, cleared in xylene, and embedded in paraffin wax for sectioning. The 4-µm thick sections were cut, mounted on glass slides, and stained with hematoxylin and eosin stain (***Bancroft and Steven, 1996***).

Preparation for Transmission Electron Microscopy (TEM):

Three renal tissues samples were immediately placed in 5% gluteraldehyde buffered at pH 7.4 with Milloning phosphate for 4 hours and subsequently fixed in 1% osmium tetraoxide for two hours .The samples were dehydrated in graded ethanol and embedded in araldite. Thin sections were stained with lead citrate and uranyl acetate, and examined with a JEOL 1010 Transmission Electron Microscope at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University Cairo.

Biochemical analysis

1-Urea and creatinine assessment in serum:

By using a commercially available spectrophotometric enzymatic kit (Thermo Trace BECGMAN, Germany) and according to ***Young (2000).***

2-Biochemical estimation of markers of oxidative stress:

MDA content was measured according to the earlier method reported **(*Zhang, 1992*).** Reduced Glutathione was assayed according to the previous reports (***Jollow et al. 1974, Carlberg and Mannervik, 1975;*** and ***Mohandas et al., 1984***): A 1.0mL supernatant of renal homogenate was mixed with 1.0mL of sulfosalicylic acid (4%). The samples were incubated at 4ºC for at least 1 h and then centrifuged at 1200x g for 15 min at 4º C. The reaction mixture contained 0.4mL of the filtered sample, 2.2mL phosphate buffer (0.1M, pH 7.4), and 0.4mL DTNB (4 mg<mL) in a total volume of 3.0mL. The yellow color developed was read immediately at 412 nm on a spectrophotometer.

SOD activity was determined according to the previous report (***Rai et al., 2006***) CAT activity was determined from the rate of decomposition of H2O2 by the reported method (***Bergmeyer et al., 1974***). Protein content in the tissue was determined by the method reported earlier (***Lowry et al., 1951***) using bovine serum albumin (BSA) as the standard.

Statistical Analysis:

The data were analyzed by using SPSS 11.0 for Windows. The significance of differences was calculated by using one-way analysis of variance (ANOVA) followed by LSD procedure for multiple comparisons. $P<0.05$ was considered statistically significant.

Aqueous extract preparation:

According to ***Abdul* (*1990) and Bako et al. (2010***). 100 g of Hibiscus sabdariffa leaves and seeds were washed thoroughly, sun dried and ground into powder. Then 2500 ml of distilled water was added. The mixtures were then shaken for ten hours with mechanical shaker and left over night. The supernatant liquid (extract) was filtered through a Whatman's No.1 filter paper. The process was repeated for complete extraction. The extract was then poured into evaporating dish to evaporate the solvent in the extract over the water bath at the temperature of 40-45ºC. A sold extract weighing 8.4 g was obtained (***Okasha et al., 2008***). The extract stored in the refrigerator at 4 ºC for further use.

3. Results

1- Light microscopic findings:

Light microscopic examination of the kidney of the control albino rat and Roselle treated rat showed that the normal renal corpuscle consisted of glomerulus and Bowman's capsule. The glomerulus was a globular network of densely packed anastmosing capillaries. The numerous nuclei in the glomerulus were those of the capillary endothelial cells, mesangial cells and podocytes. The Bowman's capsule was formed of the parietal layer characterized by its flat nuclei of the squamous cells lining it, while the visceral layer was formed of podocytes. The Bowman's space was the space between the parietal layer and the glomerular tuft **(Figs.1&2).**

The proximal convoluted tubules (PCT) appeared rounded, and were lined by a single layer of short columnar cells with indistinct cell boundaries, and spherical nuclei. The free ends of these cells had well-developed brush borders that almost fill most of the lumen **(Figs.1&2).**

The distal convoluted tubules (DCT) were lined with simple cuboidal epithelial cells that possess distinct cell boundaries and a granular cytoplasm, and spherical centrally located nuclei. The lumen of distal convoluted tubule was wider with more defined lumen **(Figs. 1&2).**

In one-month Malathion-dosed rats, revealed distinct pathological lesions. Some glomeruli were manifested by their hypertrophy probably due to their congestion and the mild proliferation of their constituent cells **(Fig. 3).** In addition other glomeruli had lost their normal circular shape and converted into shrunken, abnormally cellular and relatively vascular structures having few red blood cells and leaving rather wide urinary space **(Fig. 4).**

Intertubular extensive hemorrhagic areas were obviously noticed among the tubules (**Figs. 3&4).** Also areas of cavitations with tissue loss were easily noticed (**Fig. 5**). Moreover the lining cells of many tubules had ill-defined boundaries and contained lightly stained large vesicular nuclei or dark pyknotic nuclei. **(Figs. 3&4).** Also, the cytoplasm of the lining cells were completely degenerated and replaced by vacuolar spaces and acidophilic masses (**Figs. 3-5).**

As regard the proximal convoluted tubules, they were greatly affected if compared to distal convoluted tubules. The outer borders of the cells which lining the convoluted tubules were deteriorated, their lumina contained faintly stained casts and the brush borders of some proximal convoluted tubules were destructed (**Figs. 4&5).** Furthermore some tubules were replaced by homogenous acidophilic material or vacuoles and the lumina of these tubules became relatively small **(Fig. 4).**

Examination of longitudinal sections of the kidneys of group VI, the Malathion plus Roselle treated rat, showed that the glomerulus, the proximal and distal convoluted tubules were nearly similar to the control group except the persistent presence of an area of cellular infiltration **(Fig.6).**

2- Electron microscopic findings

Examination of ultrathin sections of the kidney of the control group revealed the usual component of the glomeruli where part of capillary loop was recognized by its fenestration **(Fig.7).** The capillaries were lined by a thin layer of fenestrated endothelium. The podocyte nucleus and its primary process gave rise to numerous secondary foot process rested on glomerular basement membrane (**Figs. 7&8).** Also, the thickness of basement membrane was uniform **(Fig.8).**

As regard the proximal convoluted tubules (PCTs), were characterized by its narrow lumen. The large cubical cells that lined it had prominent apical microvilli. The cytoplasm beneath the microvilli contained many pyknotic vesicles. The nucleus of each cell was rounded and basally located. Numerous scattered mitochondria were observed **(Fig. 9).**

In addition the distal convoluted tubule (DCTs) had many ultastrucural features in common with the proximal tubule the most striking difference were that the distal tubule lacked the prominent microvilli and the nuclei of its cells tend to bulge into the lumen with fewer mitochondria **(Fig. 10)** .

Examination of ultrathin sections of the kidney of one- month Malathion dosed rats revealed obvious signs of degeneration. Extremely narrwoing of capillary spaces with deposition of elecrtron dense material in the podocyte **(Fig. 11).** Moreover a slight increase in the thickness of the glomerular basement membrane with deposition of electron dense material was focally seen **(Fig.12)**.In addition, the mesangial cells appeared well defined with scattered electron dense fibrils were noticed **(Fig.11).**Furthermore, there was vacuolation of the podocyte cytoplasm with fusion of its foot processes **(Figs.11&12).**

As regard the ultra structures of the cells of renal proximal tubules, there were partial loss of microvilli with electron pale material in lumen and the membranous structures were observed, as well as there were swollen and pleomorphic mitochondria were a dominant feature **(Fig.13).** The Cytoplasmic bulges were seen in some distal convoluted tubular cells **(Fig.14).**

Also, there was an increase in the cytoplasmic density of some of the distal convoluted tubule cells. Another prominent signs were the extension in the length of some cells and cytoplasmic bulges toward the lumen from the apical cytoplasm. Moreover the lumen contained electron dense material **(Fig.15).**

In the other hand , ultrathin sections of the kidney of Malathion pulse Roselle confirmed the light microscopic finding whereas it relieved normal renal ultra structures, normal glomeruli with normal

capillary lumen ,normal podocyte and mesangial cells. Moreover the foot processes of podocyte were rested on the glomerular basement membrane similar to the control group **(Figs. 15&16).** Furthermore the proximal convoluted tubules had normal nucleus, microvilli and mitochondria. Also the distal convoluted tubules appeared with clear lumen and normal nucleus **(Fig.17)**.In spite of these signs of improvement the fenestrations could not be detected **(Figs.15&16).**

3-Evaluation of the organ Weights:

No statistically significant difference in the absolute kidney weight has been observed between the control and ROS-treated group (P>0.05). However, absolute kidney weight showed significant increase in Malathion-treated groups compared to control group (P<0.001). Moreover there was significant decrease in the absolute kidney weight for Malathion+ Ros–treated group when compared to Malathion treated group (P<0.001). When the absolute kidney weight for Malathion+ Ros-treated group was compared to control group, no statistically significant changes were observed (P>0.05). ***(Table 18).***

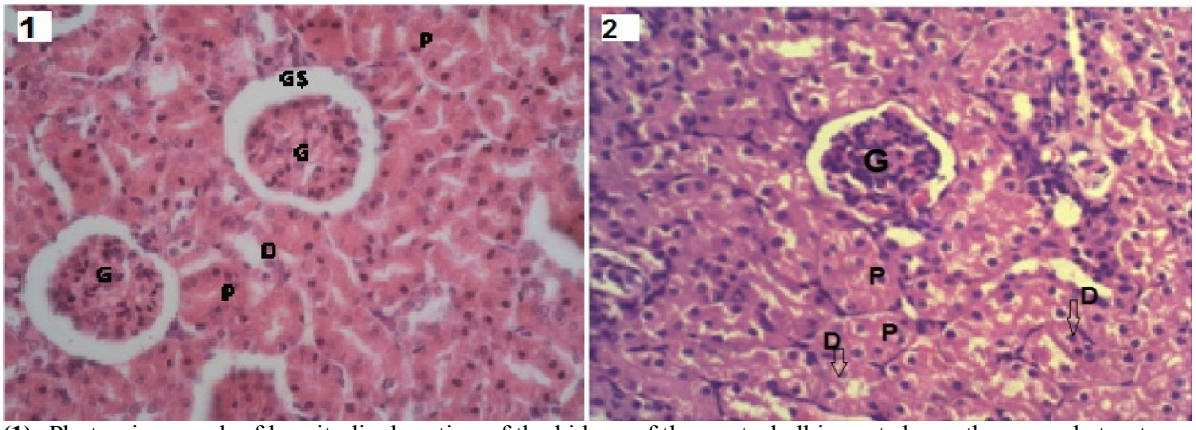


Fig. (1): Photomicrograph of longitudinal section of the kidney of the control albino rat shows the normal structure of glomerulus **(G),** glomerular space **(GS)**, proximal convoluted tubules **(P)** and distal convoluted tubules **(D). (Hx. & E.; X400).**

Fig.(2): Photomicrograph of longitudinal section of the kidney of the albino rat exposed to Roselle only shows the glomerulus **(G)**, proximal convoluted tubules **(P)** and distal convoluted tubules **(D)** are normal similar to the control group **. (Hx. & E.; X400).**

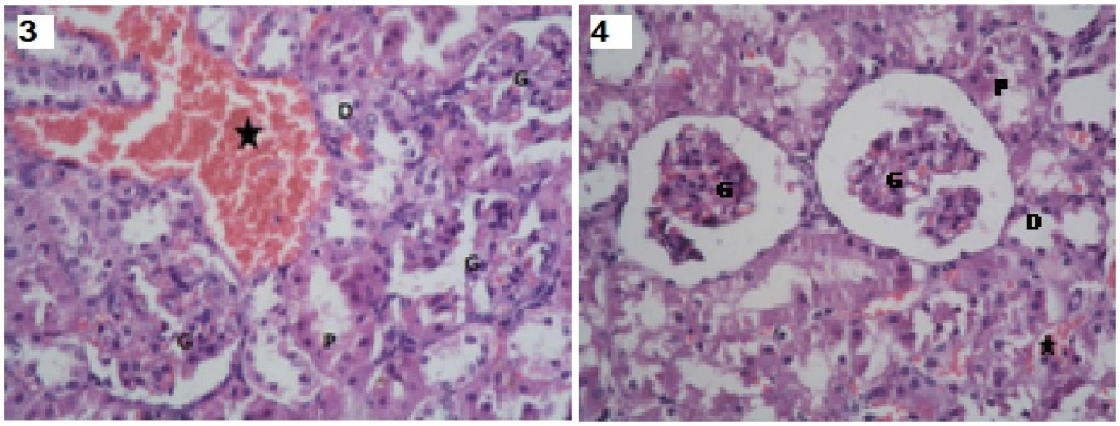
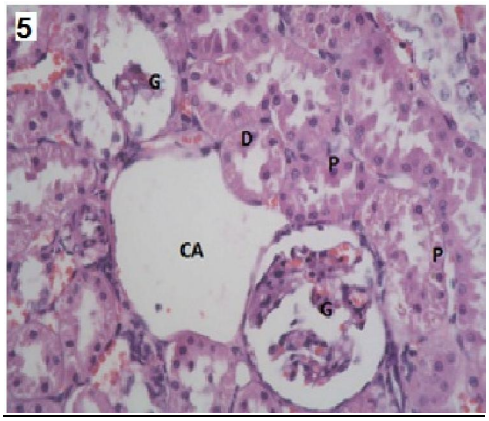


Fig.(3): Photomicrograph of longitudinal section of the kidney of the Malathion treated rat shows: presence of glomerulus hypertrophy (G), vacuolation of cytoplasm of the lining cells of both the proximal convoluted tubules (P) and distal convoluted tubules (D).Notice the presence of area of hemorrhage **(*). (Hx. & E.; X=400).**

Fig. (4): Photomicrograph of longitudinal section of the kidney of the Malathion treated rat shows the presence of: glomerular atrophy **(G) ,** the proximal convoluted tubules **(P)** contained large vesicular nuclei and the distal convoluted tubules **(D)** contained dark pyknotic nuclei. Notice the presence of an area of hemorrhage **(*). (Hx. & E.; X=400).**



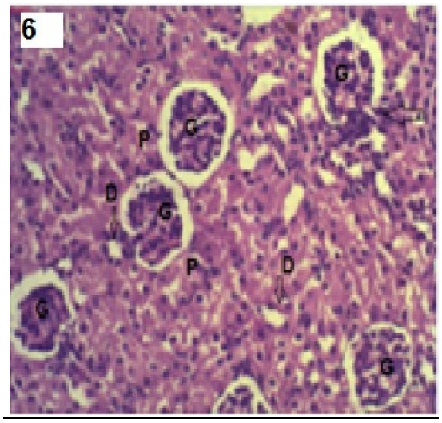


Fig .(5): Photomicrograph of longitudinal section of the kidney of the Malathion treated rat shows the atrophied glomerulus **(G)** and the proximal convoluted tubules lumen **(P)** contain casts . Notice the presence of an area of cavitation (CA). **(Hx. & E.; X=400).**

Fig. (6) :Photomicrograph of longitudinal section of the kidney of the Malathion plus Roselle-treated rat shows that the glomerulus **(G) ,** the proximal convoluted tubules **(P)** and distal tubule are normal in shape except for the presence of an area of cellular infiltration (arrow) **(Hx. & E.; X=400).**

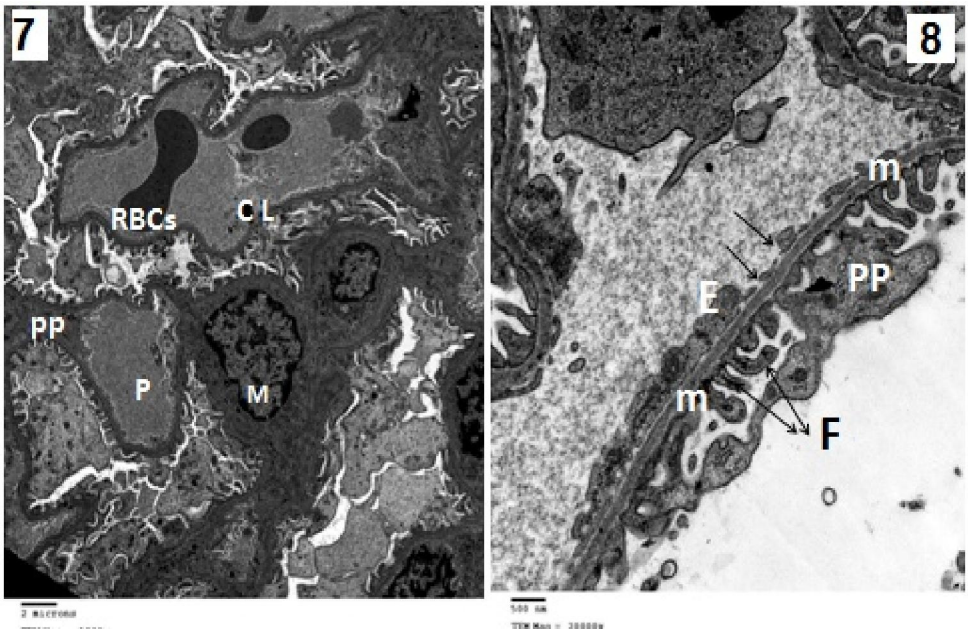


Fig. (7): Electron micrograph of ultrathin section of the control group revealed the usual component of the glomeruli showing: normal part of capillary lumen **(CL)** which contains red blood cells **(RBCs)**. Notice the normal podocyte **(P)** and its primary process **(pp). (TEM. Mag =5000)**

Fig. (8): Electron micrograph of ultrathin section of the control group of the previous figure shows normal foot processes of podocyte process **(pp)** rest on the glomerular basement membrane **(m)** which lined by capillary endothelium **.** Notice the fenestrations **(arrows)** and foot processes **(F). (TEM Mag = 20000).**

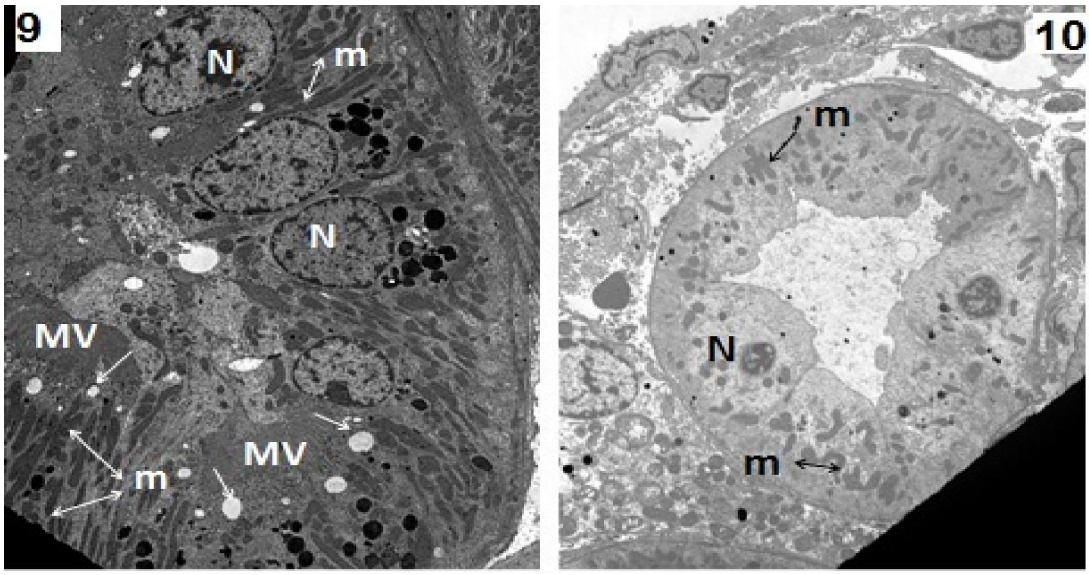


Fig. (9): Electron micrograph of ultrathin section of the control group shows normal proximal convoluted tubules shows normal nucleus **(N)**, microvilli **(Mv)** ,mitochondria **(m)** and pinocytic vesicles **(arrows). (TEM Mag =4 000**)
Fig.(10): Electron micrograph of ultrathin section of the control group shows normal distal convoluted tubules shows normal shaped nucleus **(N)** with few mitochondria **(m). (TEM Mag =4 000**)

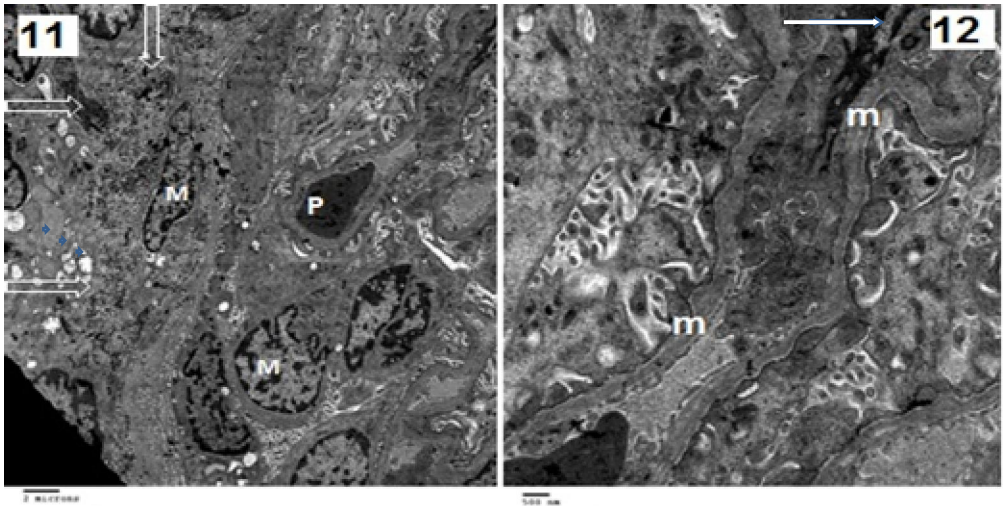


Fig.(11) : Electron micrograph of ultrathin section of the kidney of the Malathion treated rat shows extrem narrowing of capillary spaces with presence of elecrtron dense material in podocyte **(P)** and scattered electron dense fibrils **(arrows). (TEM Mag = 4000)**
Fig.(12) : Electron micrograph of ultrathin section of the previous figure shows thickning of basment membrane **(m)** with deposition of elecrtron dense material (arrow). **(TEM Mag=15000).**

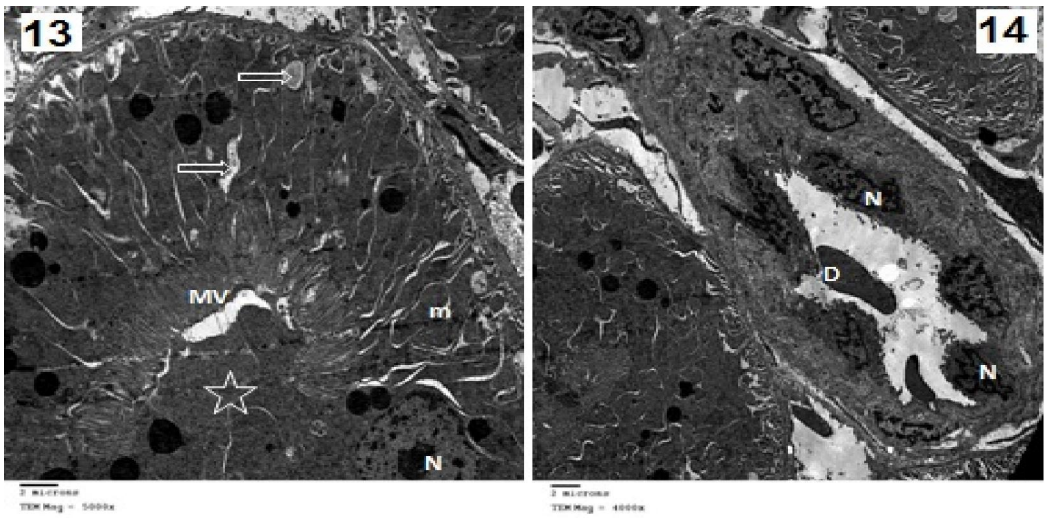


Fig. (13): Electron micrograph of ultrathin section of PCT of Malathion dosed rat kidney shows: the presence of normal nucleus **(N)** and swollen mitochondria **(m) .**Notice the presence of membranous structures **(arrows)** and electron pale material in the lumen (*) **(TEM Mag = 5 000).**
Fig. (14): Electron micrograph of ultrathin section of (DCT) of Malathion dosed rat kidney shows the normal nucleus **(N)** and electron dense material **(D)** in its lumen.**(TEM Mag = 4000).**

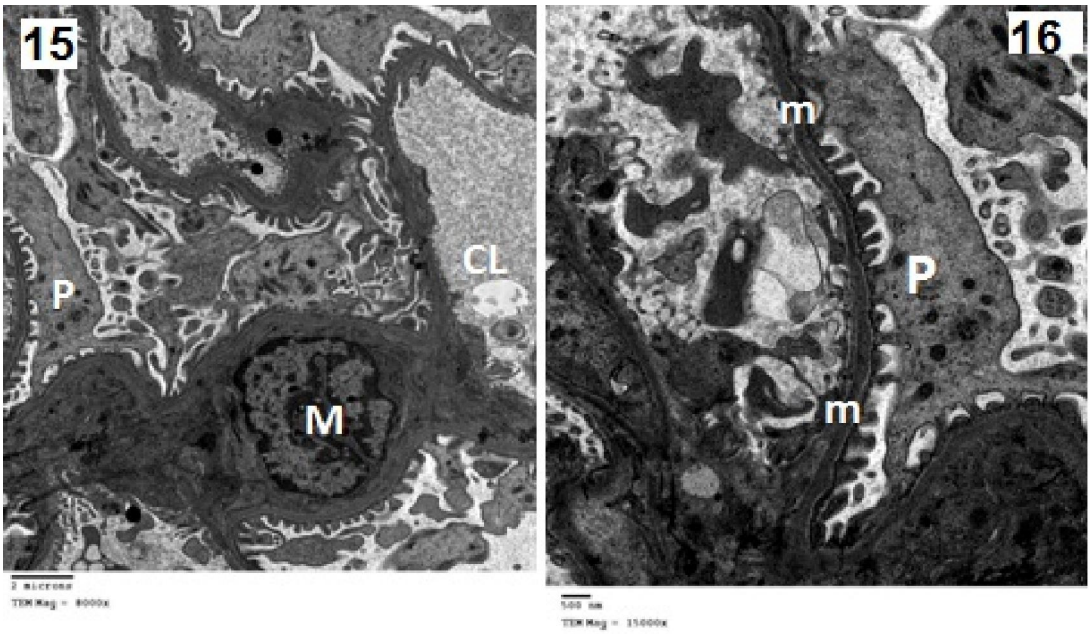


Fig.(15) :Electron micrograph of ultrathin section of of Malathion plus Roselle shows the usual component of the glomeruli showing: normal part of capillary lumen **(CL)** Notice the normal podocyte **(P)** and mesangial cells **(M)**.
TEM Mag = 3000).
Fig. (16): Electron micrograph of ultrathin section of Malathion plus Roselle shows normal foot processes of podocyte**(P)** rest on the glomerular basement membrane **(m). (TEM Mag =15000)**

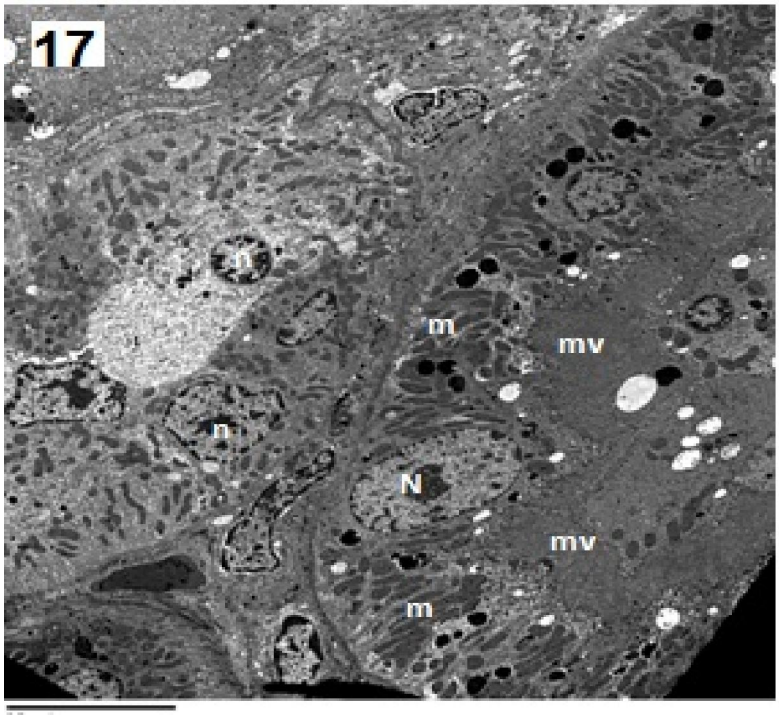


Fig. (17): Electron micrograph of ultrathin section of Malathion plus Roselle shows part of (PCT) with normal nucleus **(N),** microvilli **(mv)** and mitochondria (to the right side of the picture). Also part of (DCT) with normal nucleus **(n)** and clear lumen (to the left side of the picture). **(TEM mag =15000).**

Table (1): the Renal function, Oxidative Status & Antioxidant Enzymes in Control, ROS-treated,Malathion treated and Malathion+ROS-treated groups

Parameters	Group (n<6)	Mean ±SD	P value		
			VS control	Vs ROS-treated	Vs Malathion - treated
Absolute Kidney weights (g)	Control	19.85±0.86			
	ROS-treated	20.17±0.45	P>0.05		
	Malathion–treated	22.05±0.54	P<0.000	P<0.000*	
	Malathion+ROS treated	20.67±0.66	P>0.05	P<0.000*	P<0.001*
Urea (mg/dl)	Control	38.3±0.675			
	ROS-treated	37.9±0.876	P>0.05		
	Malathion–treated	44.2±1.751	P<0.001*	P<0.001*	
	Malathion+ROS treated	37.9±0.994	P>0.05	P>0.05	P<0.001*
Creatinine (mg/dl)	Control	1.29±0.057			
	ROS-treated	1.28±0.034	P>0.05		
	Malathion–treated	1.43±0.048	P<0.001*	P<0.00*	
	Malathion+ROS treated	1.28±0.063	P>0.05	P>0.05	P<0.001*
Tissue MDA (nm/g of tissue)	Control	1.81±0.025			
	ROS-treated	1.61±.152	P<0.001*		
	Malathion–treated	2.13±0.131	P<0.001*	P<0.001*	
	Malathion+ROS treated	1.60±0.063	P<0.001*	P>0.05	P<0.001*
Tissue Catalase (mmol/ g of tissue)	Control	0.53±0.008			
	ROS-treated	0.53±0.067	P>0.05-		
	Malathion–treated	0.26±0.014	P<0.001*	P<0.001*	
	Malathion+ROS treated	0.50±0.063	P>0.05-	P>0.05-	P<0.001*
Tissue SOD (U/ g of tissue)	Control	28.62±0.261			
	ROS-treated	29.23±0.281	P<.015*		
	Malathion–treated	19.83±0.117	P<0.001*	P<0.001*	
	Malathion+ROS treated	23.96±0.996	P<0.001*	P<0.001*	P<0.001*
Tissue GSH (U/ g of tissue)	Control	4.28±0.071			
	ROS-treated	4.17±0.307	P>0.05		
	Malathion–treated	1.54±0.092	P<0.001*	P<0.001*	
	Malathion+ROS treated	3.85±0.128	P<0.001*	P<0.001*	P<0.001*

* The mean difference is significant at the 0.05 level.

4- Evaluation of Biochemical Results:

1-Results of the oxidative stress markers:

The activity of CAT in the Malathion-treated group was significantly (P<0.001) decreased when compared to the control animals. Treatment with the extract of *Roselle* significantly prevented decrease in the level of CAT activity compared to the Malathion treated rats (p<0.001). Renal SOD activity was decreased significantly (p<0.001) in the Malathion treated-animals compared to control group. Treatment with the extract of *Roselle* significantly elevated (P<0.001) the SOD levels as compared to the malathion-treated animals. The GSH level reduced significantly (P<0.001) along with increased in MDA concentration in the Malathion treated group as compared to the control group (p<0.001). However on treatment with *Roselle* extract, the GSH level was found to be enhanced significantly (p<0.001) and the MDA contents were reduced in (P<0.001) as compared to the Malathion treated group. These data were represented in ***Table (1).***

2- The Urea and creatinine levels:

At the end of the experiments, when the Malathion-treated group was compared with the control group, there was a significant increase in the serum urea and creatinine levels (P<0.001 for both) (Figs.19 and 20). In the Roselle + Malathion treated group there was a statistically decrease in the serum urea level and creatinine levels when compared to the Malathion-treated group (P<0.001 for both). There were statistically insignificant differences between the levels of urea and creatinine in Roselle+ Malathion- treated group when compared to the control group (p>0.05), **Table (1).**

4. Discussion

The Kidney is a target organ of the experimental animals attacked by OP compounds **(*Mansour et al., 2010*).** Malathion is used in public health, agriculture and household purposes (***Lasram et al., 2008*).** Several studies showed that Malathion caused hepatotoxicity (***Kalender et al., 2010*),** testicular toxicity (***Uzun et al., 2009***), hematotoxicity (***Durak et al., 2009; Al-Attar, 2010*),** genotoxicity **(*Giri et al., 2002*)** and nephrotoxicity **(*Al-Attar, 2010*).**

In the current study, all the examined parameters in group II (Roselle alone) were similar to the control group except that Roselle administration significantly decreased the tissue MDA activity and increased the SOD activity indicating that Roselle is safe herbal to the kidney with significant anti-oxidative stress effect. These results were in line with the results of ***Abbas et al.(2011)*** who concluded that *H. Sabdariffa* is probably a safe medicinal plant as there wasn't significant harmful change in cholesterol, triglyceride, BUN, serum creatinine and Na and K levels were observed. In addition, ***Amin and Hamza* (*2006)*** noticed insignificant changes in the weights of testes and epididymis in rats treated with *H. Sabdariffa.* Moreover, many studies indicated that Roselle is an interesting herb ingredient because its petals consist of anthocyanin pigment which has many properties corresponding to biological activities such as antioxidant activity (***Tseng et al., 1997b; Wang et al., 2000; Liu et al., 2002; Tsai et al., 2002; Ali et al., 2003***).

In the current study, light microscopic examination of the kidneys of the group III, one-month treated rat with Malathion, revealed that the golmerular tufts showed marked alterations including shrinkage or hypertrophy and congestion. Also areas of hemorrhage were obvious. These results were in consistence with the results of ***Abdel Rahman and Zaki* (*1992) and Afshar et al.(2008)*** who noticed deformation of the structure of the glomeruli of the cortical region in mice kidney treated with Malathion or sevin. Furthermore, ***Enan et al., (1983)*** noticed that pesticides caused degeneration followed by dilation of the proximal and distal convoluted tubules which contained hyaline casts.

Moreover, by the electron microscopic examination the results revealed that the degenerative changes appeared clearer indicated pronounced changes in the renal corpuscles .These changes including swelling appearances, decrease of urinary spaces, and highly degeneration and fibrosis of podocyte. Also, there were distortion of Bowman's capsules and vacuolar degeneration of associated tubules.

The glomerular structure was affected markedly by administration of Malathion. The presence of prominent degeneration in the cytoplasm of podocytes and fusion of pedicels with fibrosis were striking. Fusion of pedicels may indicate a toxic effect of Malathion while degeneration in glomerules may be related with the interaction of the biological membrane with Malathion during its passage through the filtration barrier. ***Bertani et al.* (*1982*)** reported that fusion of pedicels and proteinurea in kidney after administration of adriamiycin in rat were suggested to be developed as a result of loss of electrical load in podocyte pedicels.

In the current study, an increase in the thickness of the glomerular basal membrane with deposition of electron dense material may be due to the fact that all the organophosphorous pesticides (OP) are lipophilic and are known to have a strong affinity for interaction with membrane phospholipids **(*Antunes and Madeira, 1987; Datta et al., 1994)***.

Many studies reported that organo-phosphorous compounds; Malathion, may induce oxidative stress leading to the generation of free radicals and alterations in antioxidant and scavengers of oxygen-free radicals which alter structural and functional integrity of cell membrane **(*Bagchi et al., 1995*; *Poovala et al., 1999;***

Ahmed et al., 2000; Possamaia et al., 2007*; *Franco et al., 2009).

The effect of Malathion on the PCT revealed swelling of the mitochondrial membrane. It may be due to the effect of Malathion metabolites that can produce oxygen free radicals and affect configuration and active transport of the cell membrane. Moreover, the lipophilic metabolites of Malathion may impair the membrane of the mitochondria and play a role in the mitochondrial dysfunction. ***Verma et al.* (*1978*)** reported that pesticides caused alteration of the membrane configuration and inhibition of Mg, Na, K and ATPase in concomitant with a high concentration of aldrin and dieldrin in homogenate of kidney tissue. The membranes of mitochondria are rich with unsaturated fatty acids which are sensitive to free radicals **(*Mc Cord et al., 1978*).**

In the current study, there were also many tubular degenerative signs like casts in the proximal convoluted tubular lumen and the presence of cytoplasmic bulges which were seen in some degenerative distal convoluted tubular cells. These signs suggested the presence of lipid peroxidation and production of free radicals which destruct the lipid and protein structure of intracellular membranes and lyses of cytoplasm **(*Tos-luty et al., 2003 and Saadi et al., 2008).***

All the histopathological changes in the current study were in concomitant with biochemical changes as, there were significant increase in the serum urea and creatinine levels in Malathion treated group. These results were in line with the results of ***Kerem et al.* (*2007*)** and ***Al-Attar* (*2010*)** who reported significant changes of kidney hemato-biochemical indices including statistically increased levels of creatinine, urea and uric acid, and decreased levels of total protein and albumin concentrations in all animals treated by Malathion. Also, ***Husain* (*2004*) and *Mansour et al.* (*2010*)** reported that pesticides can alter plasma urea, uric acid and creatinine levels as a result of the impairment of the glomerular function and tubular damage in the kidneys.

***Mora et al.* (*2003*) *and Yearout et al.* (*2008*)** considered the creatinine level as a good risk marker for chronic renal insufficiency.

This impairment in the kidney functions may be due to the tubular degeneration as a result of oxidative stress which induced by Malathion and evidenced by decreased CAT and SOD activities with significant reduction in the GSH level along with increased MDA concentration in the in renal tissues. Furthermore, in the present study, the presence of oblivious injuries to the membranous structures in the cytoplasm of some cells of the proximal convoluted tubule may reflect the probable injury caused by oxidative radicals.

Administration of aqueous extract of Roselle prior to Malathion resulted in a significant alleviation of the kidney injuries evidenced by a reduction of the absolute kidney weights, biochemical indices and improvement of the histological picture toward the normal. There was a statistically significant decrease in the weight of the kidney indicating improvement of the sign of degeneration and inflammation of the kidney. Moreover, the serum urea and creatinine levels were decreased toward the normal control levels. Furthermore, the glomeruli appeared within normal size with normal capillary lumen and podocyte. The mesangial cells showed normal foot processes of podocyte which rested on the normal glomerular basement membrane nearly similar to the control group. In addition, the proximal convoluted tubules had normal nucleus, microvilli and mitochondria. The distal convoluted tubules had a normal nucleus and clear lumen.

These improvements attributed to the antioxidant protective effect of Roselle and its scavenger effect. Similar histopathological findings, by using aqueous extract of Roselle, were reported by ***Kalyan et al. (2009) and Josiah et al. (2010) .Also*** these improvement are evident in the current study by increased CAT and SOD activities along with decreased MDA and increased GSH levels in the renal tissues. These results were in consistence with the results of many study that revealed that the extract of Roselle ingnificantly decreased the leakage of lactate dehydrogenase, formation of MDA and liver damage induced by t-BHP (***Tseng et al., 1997b; Wang et al., 2000***) and paracetamol (***Ali et al., 2003***) and increased glutathione (***Wang et al., 2000***) in rats. The antioxidant activity of Roslle could be attributed to its phenolic contents, namely protocatechuic acid as cited by ***Liu et al. (2002)*, *Tsai and Huang* (*2004*) and *Prenesti et al.* (*2007*).** Moreover, ***Amin and Hamza (2005)*** reported that Roselle could prevent or attenuate the decrease in tissue anti-oxidant enzymes in different animal models and to provide cellular protection against oxidative stress.

Also similar results were reported by ***Tebekeme and Ibiba* (*2008*)** who reported that reduction in the levels of serum creatinine, urea and the elevation of the levels of kidney GSH and catalase in rats treated by cytotoxic drug, cisplatin, with roslle indicate that Roselle extract can reduced cisplatin induced kidney dysfunction. From their results, they suggested that the various phytochemicals extract acted synergistically to sequester the free filterable platinum hence making it less available for cellular damage.

***Fatma Gökçe and Yusuf* (*2011*)** indicated that a low dose of Malathion caused sub acute nephrotoxicity that could not be ameliorated by the antioxidant vitamins; vitamin C and E, these results may indicate more potent anti-oxidant effect of Roselle over these vitamins. Furthermore, ***Farombi and Fakoya* (*2005*)** observed that the Roselle extracts has antimutagenic activity (60% to 70%) greater than that of vitamin C.

In conclusion, the results of the current study revealed that the aqueous extracts from Hibiscus sabdariffa is considered as a safe, natural and potent antioxidant that protects the kidney from the oxidative stress induced by chronic exposure to sub-lethal dose of Malathion.

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References:

Abbas, M; Shirin, M; Patricia, K and Mohammad, GK (2011): The Effect of Hibiscus Sabdariffa on Lipid Profil Creatinine, and Serum Electrolytes: A Randomized Clinical Trial. *Gastroenterology*, 2011: 4.

Abdel Rahman, MF and Zaki, ZT (1992): Histopathological and histochemical effects of sevin on the renal and hepatic tissues of mice. *Journal of the Egyptian German Society of Zoology,* 8C:115-126.

Abdollahi, M; Mostafalou, S; Pournour mohammadi, S; Shadnia, S. (2004): Oxidative stress and cholinesterase inhibition in saliva and plasma of rats following subchronic exposure to malathion. *Comp Biochem Physiol C Toxicol Pharmacol., 137:29-34.*

Abdul, G (1990): Introduction to Pharmacognosy. University press, 1st Edn., pp: 175-200.

Afshar, S; Farshid, AA; Heidari, R and Ilkhanipour, M (2008): Histopathological changes in the liver and kidney tissues of Wistar albino rat exposed to fenitrothion. *Toxicology and Industrial Health,* 24(9): 581–586.

Ahmed, RS; Seth, V; Pasha, ST and Banerjee, BD (2000): Influence of dietary ginger (*Zingiber officinales* Rosc) on oxidative stress induced by Malathion in rats," *Food and Chemical Toxicology*, 38: 5, 443–450.

Akhtar, N; Kayani, S; Ahmad, M and Shahab, M (1996): Insecticide-induced changes in secretory activity of the thyroid gland in rats. *J. Appl. Toxicol.,* 16:5:397-400.

Al-Attar, AM (2010): Physiological and Histopathological Investigations on The Effects of α-Lipoic Acid in Rats Exposed Malathion. *Journal of Biomedicine and Biotechnology,* 2010: 1-8.

Ali, BH, Mousa, HM and Mougy, ES (2003): The effect of water extract and anthocayanins of *Hibicus sabdariffa L.* on paracetamol induced hepatoxicity in rats. *Phyto. Res*., 17 (1): 56-59.

Ali, BH; Al Wabel, N and Blunden, G (2005): Phytochemical, pharmacological and toxicological aspects of Hibiscus sabdariffa L.: a review. *Phytother Res.,* 19(5): 369-375.

Alli, MB and Salih, M (1991): Investigation of the antispasmodic potential of Hibiscus sabdariffa calyces. *J. Ethnopharmacol.,* 31: 249-257.

Amin, A and Hamza, AA (2005): Hepatoprotective effects of Hibiscus, Rosmarinus and Salvia on azathioprine-induced toxicity in rats. Life Sci., 77: 266–78.

Amin, A and Hamza, AA (2006): Effects of ginger and roselle on cisplatin induced reproductive toxicity in rats. Asian J Androl., 8: 607–12.

Amr, A; Alaaeldin, AH; Amr, K and Sayel, D (2008): Herbal extracts counteract cisplatin-mediated cell death in rat testis. *Asian Journal of Andrology*, 10: 291–297.

Antunes Madeira MC and Madeira, VMC (1987): Partition of Malathion in synthetic and native membranes .*Biochem Biophys Acta;* 901: 61–67.

Bagchi, D; Bagchi, M; Hassoun, EA and Stohs, SJ (1995): *In vitro* and *in vivo* generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. *Toxicology,* 104-129-136.

Bako, IG; Mabrouk, MA; Maje, IM; Buraimoh, AA and Abubakar, MS (2010): Hypotensive Effect of Aqueous Seed Extract of Hibiscus sabdariffa linn (Malvaceae) on Normotensive Cat. *Int. J. Anim. Sci. Veter.* Adv., 2(1): 5-8.

Bancroft, JD and Steven, SA (1996): Theory and practice of histological techniques. 4thed. Churchill Livingston, *Edinburgh, London*, 184-193.

Bergmeyer, HU, Gowehn, K and Grassel, H (1974): Methods of enzymatic analysis. *Weinheim Verlag Chemine*., 438-439.

Bertani, T; Poggi, A; Pozzani, R; Delaini, F; Sacchi, G; Thova, Y; Mecca, G; Remuzzi, G and Donati, MB (1982): Adriamycin-induced ephritic syndrome in rats: sequence of pathologic events. Lab,. 707.

Cabello, G; Valenzuela, M; Vilaxa A (2001): A rat mammary tumor model induced by the organophosphorous pesticides parathion and malathion, possibly through acetylcholinesterase inhibition. Environ Health Perspect., 109:471-9.

Carlberg, I and Mannervik, B (1975): Glutathione reductase levels in rat brain. J. Biol. Chem., 250: 5475-5479.

Datta, C; Dasgupta, JG and Sengupta, D (1994): Interaction of organophosphorous insecticides phosphamidon and Malathion on lipid profile and Acetylcholinesterase activity in human erythrocyte membrane. *Indian J. Med. Res.,* 100: 87–94.

Durak, D; Uzun, FG; Kalender, S; Ogutcu, A; Uzunhisarcikli, M and Kalender, Y (2009): Malathion-induced oxidative stress in human erythrocytes and the protective effect of vitamins C and E in vitro, *Environ. Toxicol.,* 24(3): 235–242.

Enan, EE; El Sabaawi, EA and Mashali R (1986): Enzymatic and histological studies of profenofos on white male rats. *The Egyptian Journal of Histology,* 9 (1):182.

Farombi, EO and Fakoya, A (2005): Free radical scavenging and antigenotoxic activities of natural phenolic compounds in dried flowers of Hibiscus sabdariffa L. *Mol Nutr Food Res.,*49(12):1120-1128.

Fatma Gökçe ,U and Yusuf, K (2011): Protective Effect of Vitamins C and E on Malathion- Induced Nephrotoxicity in Male Rats. *GU J Sci*., 24(2):193-201.

Franco, JL; Posser, T; Mattos, JJ (2009): Zinc reverses Malathion-induced impairment in antioxidant defences. *Toxicology Letters*, 187 (3):137–143.

Frankowski, BL (2004): American Academy of Pediatrics guidelines for the prevention and treatment of head lice infestation. Am J Manag Care;10 suppl:S269-72.

Gallo, M J and Lawryk, NJ (1991): Organic phosphorus pesticides. Handbook of Pesticide Toxicology; Hayes Jr., W. J.; Laws Jr., E. R.,Eds.; Academic Press, Inc.: San Diego,917-1123.

Galloway, T and Handy R, (2003): Immunotoxicity of organophosphorous pesticides. *Ecotoxicology*, 12:345-63.

Gervais, JA; Luukinen, B; Buhl, K and Stone, D (2009): Malathion Technical Fact Sheet; National Pesticide Information Center, Oregon State University Extension Services.

Giri, S; Prasad, SB; Giri, A and Sharma, GD(2002): Genotoxic effects of malathion an organophosphorous insecticide, using three mammalian bioassays in vivo. Mutat.Res., 514:223 - 231.

Hazarika, A; Sarkar, SN; Hajare, S; Kataria, M and Malik, JK (2003): Influence of Malathion pretreatment on the toxicity anilofos in male rats: a biochemical interaction study. *Toxicology*, 185: 1-8.

Herrera-Arellano A; Flores-Romero, S; Chávez-Soto, MA and Tortoriello, J (2004): Effectiveness and tolerability of a standardized extract from Hibiscus sabdariffa in patients with mild to moderate hypertension: a controlled and randomized clinical trial. Phytomedicine; 11: 375–82.

Husain, K; Whitworth, C and Rybak, LP (2004): Time response of carboplatin-induced nephrotoxicity in rats. *Pharmacol. Res.,* 50: 291-300.

IARC, C (1983): Monograph on the evaluation of carcinogenic risk of chemicals to man .Miscellaneous pesticides. *International Agency for research on cancer,*30. Lyon France.

John, S; Kale M; Rathore, N (2001): Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. *J Nutr Biochem*., 12:500-4.

Jollow, DJ; Mitchell, JR; Zampaglione, N; Gillete, JR (1974): Bromobenzene induced liver necrosis: Protective role of glutathione and evidence for 3,4 bromobenzene oxide as the hepatotoxic intermediate. Pharmacology, 11: 151–169.

Josiah, SJ; Omotuyi O; Oluyemi, KA; Ezea, UI; Uhunmwangho, ES (2010): Protective role of aqueous extract of Hibiscussabdariffa (calyx) against potassium bromate induced tissue damage in wistar rats. *African Journal of Biotechnology,* 9 (21): 3218-3222.

Kalender, S; Uzun, FG; Durak, D; Demir, Fand Kalender, Y (2010): Malathion-induced hepatotoxicity in rats: The effects of vitamins C and E. Food Chem .*Toxicol*., 48: 633–63.

Kalyan, SB; Christeana, AJ; Sayma, BS; Selvakumar, S and Sundara SK (2009): Antilithiatic activity of of Hibiscus Sabdariffa on ethylene glycol-induced lithiasis in rat. *Natural Prodect Radiance,* 8(1): 43-47.

Kamrin, MA (1997): Pesticide Profiles: Toxicity, Environmental Impact, and Fate; Lewis Publishers: New York: 191-195.

Kerem, M; Bedirli, N; Gürbüz, N; Bedirli, A; Akkaya, T; Ekinci, Ö; Sakrak, Ö and Pasaoglu, H (2007): Effects of acute fenthion toxicity on liver and kidney function and histology in rats. *Turkish Journal of Medical Sciences.*, 37(5):281–288.

Lasram, MM; Annabi, AB; Rezg, R; Elj, N; Slimen, S; Kamoun, A; El-Fazza, S and Gharbi, N (2008): Effect of short-time malathion administration on glucose homeostasis in Wistar rat. *Pestic Biochem. Phys.,* 92: 114-119.

Lin, HH; Chen, JH; Kuo, WH and Wang, CJ (2007): Chemo preventive properties of Hibiscus sabdariffa L. on human gastric carcinoma cells through apoptosis induction and JNK/p38 MAPK signaling activation. *Chem Biol Interact.,* 165 (1):59-75.

Liu, C; Wang, J; Chu, C; Cheng, M and Tseng, T (2002): In vivo protective effect of protocatechuic acid on tert-butyl hydroperoxide-induced rat hepatotoxicity. *Food Chem Toxicol.,* 40: 635–41.

Lo , CW; Huang, HP; Lin, HM; Chien, CT and Wang, CJ (2007): Effect of Hibiscus anthocyanins-rich extract induces apoptosis of proliferating smooth muscle cell via activation of P38 MAPK and p53 pathway. *Mol Nutr Food Res.,* 51(12):1452-1460.

Lowry, OH; Rosebrough, NJ; Farr, AL and Randal, RJ (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem*., 193: 265-275.

Mansour, SA and Mossa, AH (2010): Oxidative damage biochemical and histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. *Pestic. Biochem. Phys.,* 96: 14-23 .

Massoulie, J and Bon, S (1982): The molecular forms of cholinesterase and acetylcholinesterase in vertebrates. *Annu. Rev. Neurosci*., 5: 57-106.

Mc Cord, JM and Fridovich, I (1978): The biology and pathology of oxygen radicals. *Ann. Intern. Med.*, 82: 122-127.

Mohandas, J; Marshall, JJ; Duggin, GG; Horvath, JS and Tiller, D (1984). Differential distribution of glutathione and glutathione related enzymes in rabbit kidney: possible interactions in analgesic neuropathy. *Cancer Re*., 44: 5086-5091.

Mora, LO; Antunes, LM; Francescato, HD; Bianchi, ML (2003): The effects of oralglutamine on cisplatin-induced nephrotoxicity in rats. *Pharmacological Research,* 47 (6): 517-522.

Morton, JF (1998): Roselle. In: fruits of warm climate. Dowling, C. F. (Ed). *Media Inc. Greensboro, NCP*: 281-286.

Okasha MM; Abubakar, MS and Bako, IG (2008): Study of the effect of Aqueous *Hibiscus sabdariffa s*eeds extract on serum prolactin level of lactating female albino rat**.** *European Journal of scientific research*, 22(4):575-583.

Olvera-García, V; Castaño-Tostado, E; Rezendiz-Lopez, RI et al., (2008): Hibiscus sabdariffa L. extracts inhibit the mutagenicity in microsuspension assay and the proliferation of HeLa cells. *J Food Sci*., 73(5):75-81.

Poovala, VS; Huang, H and Salahudeen, AK (1999): Role of reactive oxygen metabolites in organophosphate-bidrin-induced renal tubular cytotoxicity. *J. Am. Soc. Nephrol.*, 10:1746-1752.

Possamaia, FP; Fortunatob, JJ; Feierb, G ; Agostinhob, FR ; Quevedob, J; Wilhelm Filhoc, D and Dal-Pizzola F (2007): Oxidative stress after acute and sub-chronic malathion intoxication in Wistar rats Environmental Toxicology and Pharmacology, 23 (2): 198-204.

Prenesti, E; Berto, S; Daniele, PG and Toso, S (2007): Antioxidant power quantification of decoction and cold infusions of Hibiscus sabdariffa flowers. Food Chem., 100: 433–8.

Rai, S., Wahile, A., Mukherjee, K., Saha, B.P. and Mukherjee, P.K. (2006): Antioxidant activity of Nelumbo nucifera [sacred lotus] seeds. *J. Ethnopharmacol*., 104: 322-27.

Reigart, JR and Roberts, JR (1999): Organophosphate Insecticides. Recognition and Management of Pesticide

Poisonings, 5th ed.; U.S Environmental Protection Agency, Office of Prevention, Pesticides and Toxic Substances, Office of Pesticide Programs, U.S. Government Printing Office: Washington, DC, pp 34-47.

Resendiz-Lopez, I; Loara-pina, B and Castano-Tostado, E (1998): Antimutagenicity of natural phenolics compounds in dried flowers from Hibiscus sabdarafa L. against1-nitropyrene. *On line:File://A:\Hib5.htm*

Rossi, A; Serraino, I and Dugo, P (2003): Protective effects of anthocyanins from blackberry in rat model of acute lung inflammation. *Free Radical Res.* ,37(8): 891-900.

Saadi, L; Lebaili, N and Benyoussi, M(2008): Exploration of cytotoxic effect of malathion on some rat organs structure. Communications in Agricultural and Applied Biological Sci-nces, 73(4): 875–881.

Tebekeme, O and Ibiba, FO (2008): The effect of Hibiscus sabdariffa calyx extract on cisplatin-induced tissue damage in rats .*Biokemistri*, 20 (2): 47-52.

Tos-luty, S; Obuchowska-Przebirowska, D; Latuszynsxa, J; Tokarska-Rodak, M and Haratym Maj, A (2003): Dermal and oral toxicity of malathion in Rat. *Ann Agric Environ. Med*., 10: 101-106.

Tsai PJ and Huang HP (2004): Effect of polymerization on the antioxidant capacity of anthocyanins in Roselle. *Food Res Intern*., 37: 313–8.

Tsai, PJ; McIntosh, J; Pearce, P; Camden, B and Jordan BR (2002): Anthocyanin and antioxidant capacity in Roselle (Hibiscus sabdariffa L.) extract. *Food Res. Intern.,* 35: 351–6.

Tseng, T; Kao, ES; Chu, CY; Chou, FP; LinWu, HW and Wang CJ (1997 a): Hisbiscus protocatechuis acid protects against oxidative damage induced by tert-butyl hydroperoxide in rat primary hepatocytes. *Chem. Biol. Int.,* 101: 137-148.

Tseng, T; Kao, ES; Chu, CY; Chou, FP; LinWu, HW; Wang CJ (1997 b): Protective effects of dried flower extracts of Hibiscus sabdariffa L. against oxidative stress in rat primary hepatocytes. *Food Chem Toxicol.,* 35: 1159–64.

Tseng, TH; Hsu, JD; Lo, MH; Chu, CY; Chou, FP and Huang, CJ (2005): Inhibitory effect of Hibiscus Protocatechuic acid on tumor promotion in mouse skin. *Cancer Lett.,* 126(2): 199.

Uzun, FG; 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.

Verma, SR; Gupta, AK; Bansal, SK and Dalela, RC (1978): In vitro disruption of ATP dependent active transport following treatment with aldrin and its epoxyanalog dieldrin in a fresh water teleost. *Labeo rohita. Toxicolgy* ,11: 193-201.

Wang, CJ; Wang, JM; Lin, WL; Chu, CY; Chou, FP; Tseng, TH (2000):Protective effect of Hisbiscus anthocyanins against tert-butyl hydroperoxide-induced hepatic toxicity in rats. *Food Chem Toxicol.;*38: 411–6.

Yang, H; Lauv, S and Sinclair, DA (2006): Nampt/PBEF/Visfatin: a regulator of mammalian health and longevity. *Exper. Geront.,* 41:718-726.

Young, DS (2000): Effects of drugs on clinical laboratory tests . 5th edition . AACC Press .

Zeid, MM ; El-Barouty, G ; Adbdel-Reheim, E; Blancato, J; Dary, C; El-Sebae, AH and Saleh, M(1993): Malathion's disposition in dermally and orally treated rats and its impact on the blood serum acetylcholine esterase and protein profile. *J. Environ.Sci. Health, Part B*, 28 (4): 413-430.

Zhang, XZ (1992): Crop Physiology Research Methods. *China Agricultural Press Beijing*, 131:207.

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Changed-transcriptional activity of retrotansposons induced by implantation of low-energy ion beam effected the expression of genes adjacent to retrotransposons

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Abstract: In order to study the expression profiles of the retrotransposons in rice implanted by low-energy ion beam and the effects on adjacent genes to these retrotransposons, we analyzed expression features of the retrotransposons in rice with exposure to the N^+ ion beam implantation (6×10^{17} N^+/cm^2), using the Agilent Rice Oligo Microarray (4×44K) Genome Array. The results showed that there were 43 probe sets in chip, 4 out of these transcripts were up or down-regulated (≥2fold), including the *gag*, *pol*, and *int*. These four transcripts were heterogeneous to the other members in the family by clustering analysis. We also found that this differential expression effects the genes expression were up 1MB to down 1MB from the differentially expressed retrotranscription ESTs, representing the same up or down regulated case. These findings suggested that retrotransposons in rice were related to the response to N^+ ion-beam implantation through the regulation of their adjacent genes.
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Key words: rice, retrotransposon, N^+ beam irradiation, EST, gene chip

Introduction

Chip technology is of high efficiency dozens thousands of times than conventional methods, and thousands of genes can be analysed in parallel test[1], so it is a powerful tool for DNA sequence and information of gene expression analysis[2]. Gene chip technology has been used in medical diagnostics, drug screening, the gene expression measurement, environmental monitoring, crop pest and detection etc., showing a very broad application prospect[3].

Retrotransposons are a class of mobile genetic factors which are widely distributed in eukaryotes. Retrotransposons encode many proteins, including the major three genes, namely *gag* (species-specific antigen), *pol* (polymerase) and *int* (integrase). The proteins encoding by the *gag* gene take part in the maturation and packaging of the retrotransposons RNA, in order to make it suitable for integration into the whole genome. *Pol* gene encodes reverse transcriptase and RNase H, which is necessary for the replication and transposition of the retrotransposons. Integrase enzyme which is encoded by *Int* make the DNA state retrotransposons integrate into a new locus on chromosome[4]. Commonly, retrotransposons keep silent in plants, but some still has the potential to switch seats. Biotic and abiotic stress can induce the activity of these retrotransposons. Some studies suggest that many retrotransposons can be activated by the isolated protoplasts, tissue culture, chilling injury, and other stress[5-9].

Since Zeng-Liang Yu spearheaded used the low-energy nitrogen ion beam irradiation to study the biological effects on crop seeds, the low-energy N^+ beam irradiation treatment has become an important method for studying plant genetics and breeding, growth and development, stress response and other aspects[10]. In this study, the rice was exposed to the low-energy nitrogen ion beam irradiation, then we extracted the total RNA, by using the rice gene chip to scan the differentially expressed ESTs related retrotansposons.

1. Materials and methods

1.1 Materials

Rice cultivar Xindao-18 (*Oryza sativa L.ssp. japonica*) was obtained from the Key Laboratory of Ion Beam Bioengineering preservation, Zhengzhou University, Henan Province and the gene chip microarray was customized in Shanghai National Engineering Research Center. The equipment of the Low Energy Ion Beam implantation (UIL. 0.1512, TNV.) with the working vacuum of $2 \times 10^{-3} \sim 5 \times 10^{-3}$ MPa was purchased from the Institute of strong electricity, Russia. Both the total RNA extraction kit (Takara D312) and DNaseI (D2215) were purchased from TaKaRa Biotechnology Co., Ltd. PCR instrument for the MJ Company (PTC-100), for the rice Agilent single gene chip microarray.

1.2 Methods

1.2.1 Low Energy Ion Beam

Select the same rice seeds (all seeds are from the same plant) putting on the dishes with the embryo upturned, then were implanted by low-energy N^+

(30keV) in dose 6×10^{17} N^+/cm^2 under the vacuum (3×10^{-3} Pa). After the exposure, part of the seeds immediately were carried into artificial climate chamber at 30 ℃ in dark conditions and germinated under proper humidity, with the seeds which had not been injected as the control N^+. There are three biological replicas for every treatment, and 130 seeds for each repetition.

1.2.2 RNA extraction

Total RNA was extracted from uniform thirty individual buds in each replicate, which were planted for 96 hours, using RNA plant reagents (Tiangen Biotech)and purified by use of the RNeasy Plant Kit (Qiagen) according to the manufacturer's instruction. The digestion of DNA with DNase I (Qiagen) was included for all RNA preparations after the extractions.

1.2.3 Determination of the vigor index

The vigor index were investigated after the seeds planted 10 days using the rest of the seeds.The whole-plant were weighed out after drying 12 h at 60 ℃. then the vigor index was calculated as:

Vigor index= Germination percentage * dry weight.

1.2.4 Agilent single microarray hybridization, scanning, data acquisition and processing

The Agilent Gene Chip hybridization and data analysis were carried out by the Shanghai Biochip National Engineering Research Center, including the scanning, data acquisition and processing.

1.2.5 Quantitative real time PCR

Select an increase of the gag EST (Os02g0514000) to do real time quantitative PCR to validate microarray. 2-ΔΔCT method was used to calculate the relative expression.

1.2.6 The cluster analysis of rice retrotransposons

According to common name, the nucleotide sequences of the genes (*gag*, *pol*, and *int*) were received from the NCBI. Then the nucleotide sequence analysis had been analyzed by the ClustalX, and the phylogenetic tree was obtained by the Mega5 software with the UPGMA method.

2. Results and Analysis

2.1 The effects of the N^+ irradiation on the vigor index

Compared the average dry weigh between the irradiation treatment and control groups using the t-test, the difference was significant (P = 0.042), and the same to the germination percentage, the P values was 0.017. From Table 1, we can calculate that the average vigor index was 8.59%, 13.07%, for the irradiated and control groups, respectively. And they had the significant difference (t-test, P = 0.024). In one word, the effects of N^+ irradiation on the rice seeds germination in 10 days, dry weight and average energy index were significant.

Table 1. Two groups of rice budding 10 days after growth situation comparison

Batch processing	Germination ratio（%）	Dry weight (g)	Vigor index（%）
Control 1	76	0.11	8.36
Control 2	73	0.14	10.22
Control 3	72	0.10	7.20
6×10^{17} N^+/cm^2-1	80	0.18	14.40
6×10^{17} N^+/cm^2-2	80	0.16	12.80
6×10^{17} N^+/cm^2-3	80	0.15	12

2.2 Real-quantitative PCR

During the three samples, the relative expression levels of the Os02g0514000 at 6×10^{17} N^+ / cm^2 were 2.5, 2.0, 3.2, respectively, showing the increasing performance, and consistent with the results of the chip.

2.3 Screening the differential expression of retrotransposons EST of the rice after the low-energy N^+ beam irradiation treatment using gene chip

Compared the differential expression of retrotrans -posons between the N^+ beam irradiation and control samples, more than 2-fold differentially expressed in the probe (Table 2) were filtered out. And the expression of samples with the exposure to N^+ beam irradiation was up-regulated mostly.

There were 21, 8, 14 gene probes for retrotransposon *gag*, *pol*, *int* respectively, which were detected by Chip. Compared with the control probe, all *gag* probes had 1.12 times (average value) in gene expression with the standard deviation of 0.46. As for the gene expression of the *pol*, *int* probes, the values were 1.22 (± 0.67), 1.28 (± 1.01), respectively.

Table 2. N^+ beam irradiation and control samples of rice differential expression of *gag*, *pol*, *int* gene conditions

Gene	ProbeName	FCAbsolute	regulation
gag	Os02g0514000	2.81	up
pol	Os08g0133100	2.74	up
	Os01g0116100	2.25	down
int	Os02g0309600	4.71	up

2.4 Cluster analysis for the genes (*gag*, *pol*, *int*)

In order to compare the nucleotide sequences of the probe which had a different expression between the N^+

beam irradiation treatment and control sample, cluster analysis had been done using the software named Mega5.1.

The analysis for genetic distance had been obtained based on the expression of retrotransposons, and we could see that most of the genetic distance of the *gag*, *pol*, and *int* is greater than 0.3, 0.3, and 0.2, respectively (Fig.1, Fig.2, Fig.3). For the *gag* probe (Fig.1), the retrotransposons (Os02g0514000) which have different expression, belongs to this class. But the retrotransposons (*pol* probe; Os08g0133100) which also had different expression clusters as a single category, and others having the same expression multiples mass as a class (Fig.2). And for the *int* probe, there have the similar results as the *pol* probe. The important was strong sequence heterogeneitythat in the three probes. These results suggested that some specific-retrotransposons take part in response to the low-energy ion beam irradiation under this dose, but few in number.

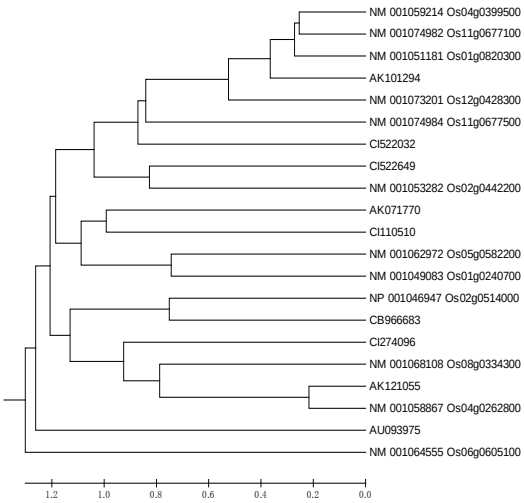

Fig.1 N^+ beam irradiation compared with control samples of rice expression *gag* probe dendrogram

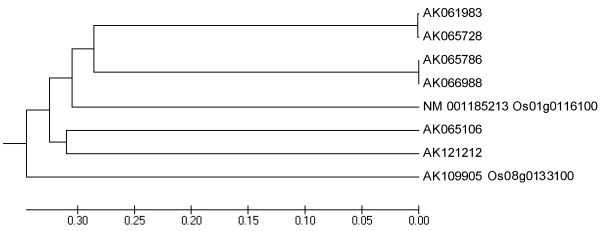

Fig.2 N^+ beam irradiation compared with control samples of rice expression *pol* probe dendrogram.

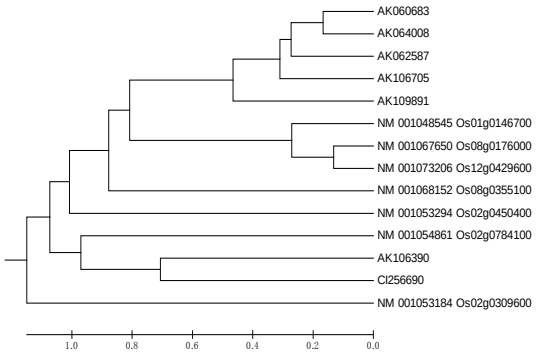

Fig.3 N^+ beam irradiation compared with control samples of rice expression *int* probe dendrogram

Through the NCBI, we found that Os02g0514000 located in between 18615320 and 18618386 on the second chromosome of the Oryza sativa (japonica cultivar group) genomic DNA. AK109905 (Os08g 0133100; short arm = 1844893, long arm = 18466) located in chromosome 8 (same genomic DNA). Os02g0309600 also had been found in chromosome 2 (same genomic DNA) between 12154821 and 12157317, and in Os02g0514000 upstream.

2.5 Analysis for the probes with differentially expressed located with 1MB chromosomal

Genetic analysis for the retrotransposons with differential expression had been done, and the probes had more than 2.7 times in different expression had been selected. Then we also compared the probes having more than 1.7 times differential expression which located within 1MB of the chromosomal. We got the results through the NCBI as follows (Table 3,4).

The genes with different expression compared with control had been found located on the retrotransposons (Os02g0514000, Os08g0133100) probe (with in 1 MB) (Table 3, Table 4). For *gag* probe (Table 3), the expression of the upstream genes were down, implying the gag may up or down modulate the gene expression of the up or down-stream gene. As for Os08g0133100 (Table 4), the upstream probes were down, while the downstream probes mostly were up, suggesting *pol* gene may have different effects on the adjacent genes (with 1MB; up-regulated for downstream gene and down-regulated for upstream).

3. Discussion

Retrotransposons are widespread presence in the plant genome, and play an important role in the genome structure, evolution and function. Studies have shown that the genes near or located within the retrotransposon may have the potential transposon, when these retrotransposons were activated by certain

stimulation, this function can cause genetic variation. Whole rice genome draft sequence reveals that retrotransposons does not eliminate but exist with no active form. Inside a gene or genes into the nearby retrotransposons may affect the time of transcription and transcription model of the adjacent genes to control their expression or silence[11]. Kashkush K. et al. had reported that Wis2-1A in the new synthetic hexaploid wheat had a high activity and stability of expression. Transcriptional activation of Wis 2-1A could have far-reaching effects on adjacent genes, when induced, the adjacent genes transcript and shape chain or antisense strand, resulting in the corresponding gene expression or silencing[12]. In this study, we took the rice as materials and used the low-energy N^+ beam irradiation to study the differences in the expression of the retrotransposon-related EST and modulation. Microarray analysis revealed that differentially expressed genes located on the chromosomal (with 1MB) of the retrotransposon *gag* probe (Os02g0514000) with differential expression, suggesting that the differential expression of the retrotransposons EST might modulate the upstream or downstream genes (with 1MB) after the N^+-beam irradiation treatment. And for the pol probe, there also existed differentially expressed genes with less than 1MB chromosomal location, and down or up for the upstream, downstream probes, respectively.

All these suggested that differential expression of pol might down-regulated for the 1MB upstream genes and played the up-regulated role for downstream genes. So the increase of part of the retrotransposon EST expression may extend their probability of transposition, strengthen the regulation of certain genes (for upstream and downstream of the gene), and for its upstream and downstream gene expression there is a certain regularity. In summary, under the low-energy ion beam irradiation treatment, the retrotransposons have effects on the expression (increase or decrease), at least play the role of gene regulation. Of course, further studied about the accurate interpretation for these phenomena need to be researched.

Table 3. Differentially expressed the *gag* probe chromo- some position within 1Mb differentially expressed probe

Probe Name	Chromosome position	p-value	FCAbsolute	Regulation
Os02g0510400	upstream 18342477 -18343176	0.005	2.13	down
Os02g0512400	upstream 18514080 -18515103	0.009	1.73	down
Os02g0517700	downstream 18824891-18825982	0.202	1.84	down
Os02g0518400	downstream 18857193-18862864	0.070	2.95	up

Table 4. Differentially expressed the *pol* probe chromo- some position within 1Mb differentially expressed probe

Probe Name	Chromosome position	p-value	FCAbsolute	regulation
Os08g0122700	upstream1578794-1582551	0.012	2.30	down
Os08g0127900	upstream 1590894-1593153	0.194	2.25	down
Os08g0128000	upstream 1594506-1598551	0.020	2.96	down
Os08g0131100	upstream 1746781-1749181	0.023	2.17	down
Os08g0136600	downstream 2054411-2055286	0.067	2.32	down
Os08g0136700	downstream 2056871-2058397	0.011	2.48	up
Os08g0136800	downstream 2060089-2061374	0.004	1.83	up
Os08g0137300	downstream2101974-2105683	0.273	2.10	up

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Reference

1. CHANG JC, Hilsenbeck SG, Fuqua SA. Genomic approches in them anagement and treatment of breast cancer. Br J Cancer, 2005, 92(4): 618-624.
2. TSUKAHAR T, Toshifumi T, Nagasawa N, et al.

Sci Technol Adv Material, 2004, 5(3): 359-362.
3. ZHANG X Y，Liu J. The application prospects of gene chip technology. Contemporary Animal Husbandry, 2003, 6: 41-43.
4. KUMARumar A, Bennetzen J L. Plant retrotransposon. Annu Rev Genet, 1999, 33: 479-532.
5. BEGUIRISTAIN T, GRANDBASTIEN M A, PUIGDOM ENEC H P, et al. Three Tnt1 subfamilies show different stress associated patterns of expression in tobacco. Consequences for retrotransposon control and evolution in plants. Plant Physiol, 2001, 127: 212-221.
6. HIROCHIKA H. Ret rot ransposons of rice: their regulation and use for genome analysis. Plant.Mol. Bio., 1997, 35: 231-240.
7. KAL ENDAR R, TANSKANEN J, IMMONEN, et al. Genome evolution of wild barley (Hordeum spontaneum) by BARE-1 retrotransposon dynamics in response to sharp microclimatic divergence. Proc. Nati. Acad. Sci. USA, 2000, 97 (12): 6603-6607。
8. STEWARD N, ITO M, YAMA GUCHI, et al. Periodic DNA methylation in maize nucleosomes and demethylation by environmental stress. J .Biol. Chem., 2002, 277: 37741- 37746.
9. TAKEDA S, SU GIMOTO K, OTSU KI H, et al. A 132bp cisregulatory element in the LTR promoter of the tobacco retrotransposon Tto1 is involved in responsive- ness to tissue culture, wounding , methyljasmonate and fungal elicitors. Plant J, 1999, 18: 383-393.
10. YU Z L,Qiu L J, Huo Y P. Biological effects of ion implantation and Breeding research progress. Anhui agronomy journal, 1991, 18(4): 251-257.
11. CHEN Z W, Wu W R. Plant retrotransposons and their application. Heresias, 2004, 26(1): 122-126.
12. KASHKUSH K, Feldman M, Levy A A. Transcriptional activation of retrotransposons alters the expression of adjacent genes in wheat. Nat Genet, 2003, 33: 102-106.

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Effect of Different Stocking Densities on Hematological and Biochemical Parameters of Silver Carp, *Hypophthalmichthys molitrix* Fingerlings

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Abstract: The impact of rearing silver carp, *Hypophthalmichthys molitrix* to the fingerling stage under three different stocking densities was investigated depending on the hematological and biochemical parameters as indicators of general health state of fish. The present study was carried out for 12 weeks to determine the most optimum stocking density for rearing silver carp. Fish were cultured in duplicates of cement ponds under stocking densities of 3, 6 and 9 fish/m^3 as T_1, T_2 and T_3 respectively. The ponds were fertilized weekly with organic fertilizer at rate of 50 g/m^3. Results of hematological analyses showed significant increase in T_1 for values of RBCs count, hemoglobin, hematocrit and mean corpuscle volume (MCV) while the lowest values were recorded in T_3 for all these parameters. Changing the stocking density had non-significant effect on values of WBCs count, mean corpuscle hemoglobin (MCH) and mean corpuscle hemoglobin concentration (MCHC). Plasma biochemical analyses showed that increasing the stocking density caused significant increase in values of plasma glucose, total protein, albumin (A) and globulin (G) associated with significant decrease in values of cholesterol and triglycerides. The highest values of A/G ratio were recorded non-significantly in T_1 and T_3 while the lowest values were recorded significantly in T_2. Meanwhile T_1 showed a significant decrease in values of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and uric acid. Values of creatinine showed non-significant increase among treatments. The findings suggest that the most optimum condition was detected in T_1 (3 fish/m^3) where most of the studied hematological and biochemical parameters were essentially normal and within the range consistent with good fish health.

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Key words: Silver carp, *Hypophthalmichthys molitrix*, Stocking density, Hematology, Biochemical parameters.

1. Introduction:

Aquaculture is currently the largest single source of fish supply in Egypt accounting for almost 65% of the total fish production of the country. Most aquaculture production in Egypt depends on freshwater or brackish water species in the Nile delta region with tilapias, mullets and carps making up more than 97% of the total country production in 2007 (GFCM, 2010). Silver carp, *Hypophthalmichthys molitrix* is a native species in China and Eastern Siberia but has been intentionally distributed all over the world for aquaculture and to control algal blooms. It is not only utilized as human food but also appreciated by its ability to clean water reservoirs from clogging algae (FAO, 2005). Carps have advantageous biological characteristics such as wide tolerance to environmental conditions, high growth rate and efficient filter feeding (Shen, 2009).

Silver carp is a typical planktonivore that consumes diatoms, dinoflagellates, chrysophytes, xanthophytes, some green algae and cyanobacteria. In addition, detritus, conglomerations of bacteria, rotifers and small crustaceans constitute major components of their natural diet. Generally, there is no need to provide supplementary formulated diet in silver carp culture which makes its production costs lower than most other cultured species (FAO, 2005).

The researches devoted to the evaluation of some biochemical parameters in cultured fish are justified by their significance for estimating the general health condition of the animals, as well as the possible effects of food availability, stocking density and exposure to stressors (Kebus *et al*., 1992; Roche and Boge, 1996; Chen *et al*., 2004; Tintos *et al.*, 2006). Some investigations have evidenced important modifications of such indices under over-density stress in cultured cyprinids (Misaila *et al*., 2005).

Many studies were concerned with evaluation of hematological and biochemical parameters of carps in normal conditions or when exposed to stressors (Koedprang *et al*., 2002; Kopp *et al*., 2009; Nicula *et al*., 2010). Most of these studies were conducted on more than 3 years old carps but relatively few information in the literature has reported these parameters in carp fry or fingerlings such as the work of Kopp *et al*. (2011) although it is

commonly known that modification of fish blood constituents during these early stages of development could affect the fish performance in the future.

The aim of the present work was to evaluate the effects of different stocking densities on hematological and biochemical parameters of silver carp, *H. molitrix* fingerlings reared in cement ponds under normal environmental conditions.

2. Materials and Methods:

The present study was conducted during summer season, 2009 for 12 weeks at the Research Unit of Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abo-Hammad, Sharkia Governorate, Egypt.

Experimental design:

A total number of 2160 silver carp with mean body weight of 1.2 ±0.2 g/fish were divided into three treatments with stocking densities of 3, 6 and 9 fish/m^3 as T_1, T_2 and T_3 respectively. Fish were randomly distributed at the desired stocking density and acclimated for two weeks in duplicates of cement ponds each measuring 20 x 2.5 x 1.2m (length x width x water depth). The total volume of each pond was 60 m^3. Before starting the experiment all fish ponds were drained completely and then were exposed to solar radiation for 2 weeks till complete dryness. Ponds were then refilled with freshwater from Ismailia Canal (branched from the River Nile).

Fish ponds were fertilized weekly with Rigirs at rate of 50 g/m^3. Rigirs is an organic fertilizer produced by Misr El-Salam International Company for producing organic fertilizers. Rigirs consist of compressed and pelleted chicken manure also heat treated in order to be free from parasites, Salmonella, Shigella and *E. coli*.

Sampling and laboratory methods:

At the end of experiment, blood was collected from the caudal vein of 8 fish per treatment using sodium citrate as anticoagulant and examined directly for total red blood cells (RBCs) and total white blood cells (WBCs) using improved Neubauer Hemocytometer and expressed as the number of cells/mm^3. Hematocrit (Ht) was determined through microhematocrit tubes using hematocrit centrifuge for 5 min. Hemoglobin concentration (Hb) was estimated using cyanomethemoglobin method (Blaxhall and Daisley, 1973). The red cell indices; mean corpuscle volume (MCV), mean corpuscle hemoglobin (MCH) and mean corpuscle hemoglobin concentration (MCHC) were calculated using standard formulae (Coles, 1986). Blood samples were centrifuged to get plasma for the determination of glucose (Trinder, 1969), cholesterol (Allain *et al*., 1974), triglycerides (McGowan *et al*., 1983), total protein (Bradford, 1976), albumin content (Dumas and Biggs, 1972), alkaline phosphatase activity (ALP, EC. 3.1.3.1) (Bergmeyer, 1974), aspartate aminotransferase activity (AST, EC. 2.6.1.1), alanine aminotransferase activity (ALT, EC. 2.6.1.2) (Reitman and Frankel, 1957), creatinine (Henry *et al*., 1974) and uric acid (Barham and Trinder, 1972) using enzymatic-colorimetric methods by means of commercial kits (Biodiagnostic Co, Egypt). The globulin content (G) was estimated by subtracting the albumin content (A) from total protein content then A/G ratio was calculated.

Statistical analysis:

The results were expressed as mean ±S.E. Data were statistically analyzed using one-way analyses of variance (ANOVA) and significance was expressed using F-value at $P<0.05$. Duncan's multiple range test was used to evaluate the comparison between means as indicated by different case letters in a descending order A, B and C using Statistical Analysis System, version 9.1 (SAS, 2006).

3. Results:

During the experimental period all treatments showed non-significant mortality with over than 83% survival in all groups also water quality parameters remained well within limits recommended for freshwater fish culture. Results of hematological analyses are presented in Table 1. Statistical analysis showed significant decrease ($P<0.05$) in values of RBCs with the highest values recorded in T_1 and the lowest values were recorded non-significantly (Duncan's test) in T_2 and T_3. Meanwhile, changing the stocking density had non-significant effect on values of WBCs, MCH and MCHC. Values of Hb and Ht showed highly significant decrease ($P<0.01$) among treatments with the highest values recorded in T_1 and the lowest ones recorded in T_3 for both parameters. Values of MCV showed significant decrease ($P<0.05$) among treatments with significantly lower values recorded in T_3.

As indicated in Table 2, the increase in stocking density was associated with highly significant increase ($P<0.01$) in plasma glucose and highly significant decrease ($P<0.01$) in plasma cholesterol and triglycerides. The highest values of cholesterol were recorded non-significantly in T_1 and T_2 while those of triglycerides were recorded significantly in T_1.

Results of the plasma protein content (Table 3) showed highly significant increase ($P<0.01$) in values of total protein, albumin and globulin associated with the increase in stocking density. Meanwhile, values of A/G ratio showed significant difference among

treatments (P<0.05) with the highest values recorded non-significantly in T_1 and T_3 and the lowest values recorded significantly in T_2.

In case of plasma indicative parameters of liver functions (ALP, AST and ALT) (Table 4), values of ALP, AST and ALT showed highly significant increase (P<0.01) with the lowest values recorded significantly in T_1 for ALP and AST. Meanwhile, the lowest values were recorded non-significantly in T_1 and T_2 for ALT. The measured plasma creatinine and uric acid (Table 4) were used as indicators of fish kidney functions. Values of creatinine showed non-significant increase among treatments. Meanwhile, values of uric acid showed highly significant increase (P<0.01) with the highest values recorded non-significantly in T_2 and T_3 while the lowest values were recorded significantly in T_1.

Table 1: Effect of different stocking densities on hematological indices of silver carp

	RBCs ($X10^6$cells/mm^3)	WBCs ($X10^3$cells/mm^3)	Hb (g/dl)	Ht (%)	MCV (μm^3)	MCH (pg)	MCHC (%)
T_1 (3 fish/m^3)	1.04 ±0.01 A	21.33 ±1.76 A	2.97 ±0.09 A	16.32 ±1.68 A	158.61 ±16.63 A	28.62 ±0.85 A	18.66 ±4.51 A
T_2 (6 fish/m^3)	0.68 ±0.11 B	23.33 ±4.67 A	1.87 ±0.07 B	8.35 ±1.17 B	149.73 ±25.42 A	29.94 ±2.78 A	23.0 ±5.09 A
T_3 (9 fish/m^3)	0.64 ±0.08 B	17.33 ±2.91 A	1.57 ±0.09 C	4.07 ±0.46 C	81.44 ±12.57 B	24.15 ±3.14 A	38.31±12.5 A
F-value	8.30*	0.84	81.50**	32.19**	5.96*	1.51	1.58

Data are represented as means of eight samples ±S.E.
Means with the same letter in the same column for each parameter are not significantly different.
* Significant difference (P<0.05) ** Highly significant difference (P<0.01)

Table 2: Effect of different stocking densities on plasma glucose, cholesterol and triglycerides of silver carp

	Glucose (mg/dl)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
T_1 (3 fish/m^3)	72.90 ±7.43 C	174.09 ±3.06 A	181.43 ±6.99 A
T_2 (6 fish/m^3)	97.52 ±5.32 B	150.01 ±2.55 A	101.79 ±29.23 B
T_3 (9 fish/m^3)	102.33 ±10.21 A	57.72 ±5.63 B	64.17 ±3.50 B
F-value	13.94**	12.63**	11.75**

Data are represented as means of eight samples ±S.E.
Means with the same letter in the same column for each parameter are not significantly different.
* Significant difference (P<0.05) ** Highly significant difference (P<0.01)

Table 3: Effect of different stocking densities on plasma protein content of silver carp

	Total protein (g/dl)	Albumin (A) (g/dl)	Globulin (G) (g/dl)	A/G ratio
T_1 (3 fish/m^3)	3.08 ±0.27 B	1.75 ±0.18 B	1.33 ±0.13 C	1.32 ±0.13 A
T_2 (6 fish/m^3)	4.91 ±0.16 A	2.22 ±0.19 A	2.69 ±0.10 A	0.83 ±0.10 B
T_3 (9 fish/m^3)	5.54 ±0.15 A	3.39 ±0.28 A	2.15 ±0.13 B	1.58 ±0.23 A
F-value	36.23**	15.03**	33.88**	5.79*

Data are represented as means of eight samples ±S.E.
Means with the same letter in the same column for each parameter are not significantly different.
* Significant difference (P<0.05) ** Highly significant difference (P<0.01)

Table 4: Effect of different stocking densities on indicative parameters of liver and kidney functions in plasma of silver carp

	ALP (U/l)	AST (U/l)	ALT (U/l)	Creatinine (mg/dl)	Uric acid (mg/dl)
T_1 (3 fish/m^3)	53.32 ±1.92 B	16.63 ±4.28 C	14.88 ±3.17 B	0.28 ±0.12 A	2.21 ±0.19 B
T_2 (6 fish/m^3)	82.26 ±4.70 A	21.75 ±4.96 B	15.88 ±1.30 B	0.31 ±0.08 A	3.15 ±0.40 A
T_3 (9 fish/m^3)	83.76 ±5.70 A	25.13 ±6.89 A	23.25 ±2.98 A	0.35 ±0.10 A	2.82 ±0.43 A
F-value	12.72**	13.61**	10.61**	0.68	11.82**

Data are represented as means of eight samples ±S.E.
Means with the same letter in the same column for each parameter are not significantly different.
* Significant difference (P<0.05) ** Highly significant difference (P<0.01)

4. Discussion:

Fish production capacity can increase by identifying environmental factors and providing appropriate environmental conditions for the fish. During the 12 weeks experimental period of the present study, water quality parameters were within the acceptable range for freshwater fish culture as indicated by Boyd (1990) and Delince (1992) making the stocking density as the main variable affecting the hematological and biochemical parameters of the studied fish.

The physiological system of fish can be severely challenged by a variety of biological, chemical and physical factors. When the physiological tolerance limits of fish are surpassed, reproductive success, growth, activity, resistance to infectious diseases and survival can all be impaired. Stress elicits a generalized endocrine response in fish which in turn induces a suite of secondary effects including rapid mobilization of energy reserves. The secondary responses of fish to the stressors can be evaluated by measurement of secondary biochemical indicators, such as changes in hematology and plasma chemistry (Wedemeyer *et al*., 1990).

Fish hematology is gaining great attention in fish culture because of its importance in monitoring the health status of fish (Hrubec *et al*., 2000). In case of cultured species, occasionally health issues arise that necessitate clinical evaluation of the fish under such captive conditions. Lack of published species-specific normal reference ranges remains the primary reason that blood testing is not routinely performed in fish health evaluations (Mauel *et al*., 2007). The knowledge of the hematological characteristics is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes in fish (Kori-Siakpere *et al*., 2005).

Alteration of fish blood biochemistry may be indicative of unsuitable environmental conditions or the presence of stressing factors (Barcellos *et al*., 2004). Our results showed significant higher values of RBCs, Hb and Ht in the statistical evaluation of T_1 compared to the other two treatments which indicates that increasing the stocking density over 3 fish/m^3 affected consequently these relevant physiological parameters of fish. This is confirmed by the significant decrease in values of RBCs, Hb, Ht and MCV at stocking density of 6 fish/m^3 (T_2) followed by 9 fish/m^3 (T_3). Similar results have been reported by Kopp *et al*. (2010) who studied the influence of cyanobacterial water bloom on hematological and biochemical indices of two years old silver carp, *H. molitrix* although they reported higher values of normal RBCs, Hb and Ht than the present study which may be attributed to the age difference of the studied fish and changes of the experimental conditions.

According to Coles (1986) the calculated blood indices have particular importance in describing anemia in most animals. The detected non-significant differences in values of MCH and MCHC support the assumption that increasing the stocking density did not pose much challenge to the erythrocytes and did not indicate any pathological condition in the studied fish, even if the decrease is significant in values of RBCs, Hb and Ht separately. This is also confirmed by the recorded non-significant difference among values of WBCs. According to Weber and Jensen (1988) erythropoiesis and hemoglobin synthesis require long time to complete and can only be involved in long-term adaptation.

Blood glucose level has been used as an indicator of environmental stress to reflect changes in carbohydrate metabolism under stress conditions (Kavitha *et al*., 2010). Tintos *et al*. (2006) reported significant increase in plasma glucose of immature gilthead sea bream, *Sparus auratus* after short-term and long-term stress conditions. This strongly supports the present findings with the detected highly significant increase in values of plasma glucose in T_2 and T_3 which were much higher than the normal reference interval of cyprinids (63-86 mg/dl)

indicated by Nicula *et al*. (2010) and T_1 showed the optimum condition regarding this parameter.

The measurement of total protein, albumin and globulin in serum or plasma is of considerable diagnostic value in fish as it relates to general nutritional status as well as the integrity of the vascular system and liver functions (Abdel-Tawwab *et al*., 2008). According to Firat and Kargin (2010) the stress response of Nile tilapia, *Oreochromis niloticus* exposed to individual and combined mixtures of heavy metals was expressed by significant increase in glucose, total protein, albumin, AST and ALT as well as a significant decrease in cholesterol levels. This strongly supports the present findings where the significant increase in values of total protein, albumin and globulin was associated with increasing the stocking density of silver carp. However, the detected values of total protein were within the normal physiological range of cyprinids (2.10-5.76 g/dl) while values of albumin in case of T_2 and T_3 were marginally higher than the normal range of cyprinids (0.53-2.20 g/dl) indicated by Nicula *et al*. (2010).

Cholesterol is one of the structural components of cell membranes as well as the outer layer of plasma lipoproteins and is the precursor of all steroid hormones (Yang and Chen, 2003). Triglycerides function primarily in providing cellular energy and can be used as an indicator of nutritional status. In fact the greatest change in body composition is usually produced in the lipid fraction (Wallaert and Babin, 1994). The measured values of cholesterol and triglycerides showed highly significant decrease among treatments. Similar trend in cholesterol values of stressed fish was described by Zhang *et al.* (2008) in crucian carp, *Carassius auratus* and by Qiu *et al*. (2009) in silver carp, *H. molitrix* and bighead carp, *Aristichthys nobilis*. During sexual maturation lipid stores are mobilized and directioned to the gonads to sustain their development (Guijarro *et al*., 2003). This can support the present findings in T_1 and T_2 where the silver carp in this premature developmental stage had higher levels of cholesterol and triglycerides in preparation to this shift of body stores of lipid. The negative effect of stressors on fish may be indicated by a decrease in body lipid content (Svobodova *et al*., 2006). The detected values of cholesterol in T_1 and T_2 were within the normal physiological range of cyprinids (65-264 mg/dl) while it was lower than this range in case of T_3 which may be attributed to the increased metabolic rate and decreased food availability. Meanwhile, all measured values of triglycerides were within the normal range of cyprinids (68-200 mg/dl) indicated by Nicula *et al*. (2010).

AST and ALT are frequently used in the diagnosis of damage caused by pollutants in various tissues such as liver, muscles and gills (De la Tore *et al.,* 2000). It is generally accepted that increased activity of these enzymes in extracellular fluid or plasma is a sensitive indicator of even minor cellular damage in the liver of fish (Palanivelu *et al.,* 2005). AST and ALT are quantitatively important in transamination of amino acids thereby allowing interplay between carbohydrate and protein metabolism during the fluctuating energy demands of the organism in various adaptive situations (Verma *et al*., 1981). Moreover, Palikova *et al*. (2010) stated that an increased value of alkaline phosphatase indicates incorrect secretion of bile due to lower food intake which may be associated with higher stress. This coincides with the recorded increase in values of glucose, total protein, ALP, AST and ALT with increasing the stocking density of silver carp in the present study. Acclimation of fish to different environmental factors results in increased metabolic activity correlated with changes in the quality and quantity of certain enzymes involved in energy metabolism and with compensating modifications in the rate of protein synthesis (Wallaert and Babin, 1994).

AST and ALT are the most frequently tested enzymes in fish for indication of cyanobacterial toxicity. The acute toxicity of microcystins (known cyanobacterial toxin) is unlikely to occur in silver carp and chronic exposure to cyanobacteria will not be detected by changes in ALP, AST and ALT in the blood plasma (Kopp *et al*., 2011). This supports our assumption that stocking density was the main variable affecting these parameters in the present study. The detected steady increase in values of ALP, AST and ALT with increasing the stocking density of silver carp, even if this increase was non-significant in some cases, indicates the consistent stress effect of increasing the stocking density on liver functions of the fish.

Nicula *et al*. (2010) indicated acceptable reference intervals of cyprinids as 46-83 U/l, 26-54 U/l and 9-23 U/l for ALP, AST and ALT respectively. Generally, values of ALP in T_2 and T_3 only were marginally higher than normal values while values of AST in the three treatments were lower than normal. The values of ALT were normal in T_1 and T_2 but marginally higher in T_3. According to Kopp *et al*. (2011) small differences among biochemical indices in a fish population are considered normal.

Plasma creatinine and uric acid levels can be used as rough indicators of glomerular filtration rate and kidney functions (Maita *et al*., 1984). Low levels of creatinine and uric acid have no significance but increasing their values indicates several disturbances in renal system (Maxine and Benjamin, 1985).

Increased level of creatinine is an indicator of kidney damage, muscular dystrophia and physical exertion of organisms (Masopust, 1998). Our results of total protein, ALP, AST, ALT, creatinine and uric acid support the assumption that the liver and muscle tissues of silver carp were markedly affected with increasing the stocking density even with the detected non-significant increase in values of creatinine. According to Nicula *et al*. (2010) the normal physiological ranges of creatinine and uric acid in cyprinids are 0.03-0.29 mg/dl and 0.78-2.20 mg/dl respectively. The detected values of creatinine and uric acid in T_1 were marginally within the upper limits of these physiological ranges while T_2 and T_3 values were higher than the normal physiological ranges of both parameters.

In conclusion, the most optimum condition was detected in T_1 (3 fish/m^3) where most of the studied hematological and biochemical parameters were essentially normal and within the range consistent with good fish health. Moreover, measurement of RBCs count, Hb, Ht, plasma glucose, triglycerides, total protein, albumin, ALP, AST and uric acid proved to be more sensitive and reliable in the detection of even minor stress response of fish.

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References:

Abdel-Tawwab, M.; Abdel-Rahman A.M. and Ismael, N.E.M. (2008). Evaluation of commercial live bakers' yeast, *Saccharomyces cerevisiae* as a growth and immunity promoter for fry Nile tilapia, *Oreochromis niloticus* (L.) challenged *in situ* with *Aeromonas hydrophila*. Aquaculture, 280: 185-189.

Allain, C.C.; Poon, L.S.; Chan, C.; Richmond, W. and Fu, P.C. (1974). Enzymatic determination of total serum cholesterol. Clin. Chem., 20(4): 470-475.

Barcellos, L.J.G.; Kreutz, L.C.; de Souza, C.; Rodrigues, L.B.; Fioreze, I.; Quevedo, R.M.; Cericato, L.; Soso, A.B.; Fagundes, M.; Conrad, J., Lacerda, L.A. and Terra, S. (2004). Hematological changes in jundia (*Rhamdia quelen* Quoy and Gaimard *Pimelodidae*) after acute and chronic stress caused by usual aquacultural management, with emphasis on immunosuppressive effects. Aquaculture, 237: 229-236.

Barham, D. and Trinder, P. (1972). Enzymatic determination of uric acid. Analyst., 97: 142-145.

Bergmeyer, H.U. (1974). Methods of enzymatic analysis. Academic Press, New York and London. Vol.4. pp.727-771.

Blaxhall, P.C. and Daisley, K.W. (1973). Routine haematological methods for use with fish blood. J. Fish Biol., 5: 771-781.

Boyd, C.E. (1990). Water quality in ponds for aquaculture. Birmingham Publishing Co. Birmingham, Alabama, USA. pp.135-170.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

Chen, C.; Wooster, G.A. and Bowser, P.R. (2004). Comparative blood chemistry and histopathology of tilapia infected with *Vibrio vulnificus* or *Streptococcus iniae* or exposed to carbon tetrachloride, gentamicin, or copper sulfate. Aquaculture, 239: 421-443.

Coles, E.H. (1986). Veterinary clinical pathology. Philadelphia, W.B. Saunders. pp.10-42.

De la Tore, F.R.; Salibian, A. and Ferrari, L. (2000). Biomarkers assessment in juvenile *Cyprinus carpio* exposed to waterborne cadmium. Environ. Pollut., 109: 227-278.

Delince, G. (1992). The ecology of fish pond ecosystem with special reference of Africa. Kluwer Academic Puplishers, London. pp.230.

Dumas, B.T. and Biggs, H.G. (1972). Standard methods of clinical chemistry. Vol. 7. Academic Press, New York, USA. pp.175.

FAO (Food and Agriculture Organization) (2005). *Hypophthalmichthys molitrix*, Cultured Aquatic Species Information Programme. Text by Yang, N. In: FAO Fisheries and Aquaculture Department [online report]. Rome. Updated 26 July 2005. http://www.fao.org/fishery/culturedspecies/Hypophthalmichthys_molitrix/en.

Firat, O. and Kargin, F. (2010). Individual and combined effects of heavy metals on serum biochemistry of Nile tilapia *Oreochromis niloticus*. Arch. Environ. Contam. Toxicol., 58: 151-157.

GFCM (General Fisheries Commission for the Mediterranean) (2010). Synthesis of Mediterranean marine finfish aquaculture - A marketing and promotion strategy. Studies and reviews. No.88, Rome, FAO.

Guijarro, A.I.; Lopez-Patino, M.A.; Pinillos, M.L.; Isorna, E.; De Pedro, N.; Alonso-Gomez, A.L.; Alonso-Bedate, M. and Delgado, M.J. (2003). Seasonal changes in haematology and metabolic resources in the tench. J. Fish Biol., 62: 803-815.

Henry, R.J.; Cannon, D.C. and Winkelman, J.W. (1974). Clinical chemistry, principles and techniques. 2nd edition, Harper and Row. New York, USA. pp.541-553.

Hrubec, T.C.; Cardinale, J.L. and Smith, S.A. (2000). Hematology and plasma chemistry reference intervals for cultured Tilapia (*Oreochromis* hybrid). Vet. Clin. Pathol., 29: 7-12.

Kavitha, C.; Malarvizhi, A.; Kumaran, S.S. and Ramesh, M. (2010). Toxicological effects of arsenate exposure on hematological, biochemical and liver transaminases activity in an Indian major carp, *Catla catla*. Food Chem. Toxicol., 48: 2848-2854.

Kebus, M.J.; Collins, M.T. and Brownfield, M.S. (1992). Effects of rearing density on the stress response and growth of rainbow trout. J. Aquat. Anim. Health., 4: 1-6.

Koedprang, W.; Nakajima, M.; Maita, M. and Taniguchi, N. (2002). Correlation of hematology and plasma chemistry levels in silver crucian carp *Carassius langsdorfii*. Fisheries Sci., 68(4): 721-728.

Kopp, R.; Mares, J.; Palikova, M.; Navratil, S.; Kubicek, Z.; Zikova, A.; Hlavkova, J. and Blaha, L. (2009). Biochemical parameters of blood plasma and content of microcystins in tissues of common carp (*Cyprinus carpio* L.) from a hypertrophic pond with cyanobacterial water bloom. Aquacult. Res., 40: 1683-1693.

Kopp, R.; Palikova, M.; Mares, J.; Navratil, S.; Kubicek, Z.; and Zikova, A. (2011). Haematological indices are

modulated in juvenile carp, *Cyprinus carpio* L., exposed to microcystins produced by cyanobacterial water bloom. J. Fish Dis., 34: 103-114.

Kopp, R.; Palikova, M.; Navratil, S.; Kubicek, Z.; Zikova, A. and Mares, J. (2010). Modulation of biochemical and haematological indices of silver carp (*Hypophthalmichthys molitrix* Val.) exposed to toxic cyanobacterial water bloom. Acta Vet. Brno., 79: 135-146.

Kori-Siakpere, O.; Ake, J.E.G. and Idoge, E. (2005). Haematological characteristics of the African snakehead, *Parachanna obscura*. Afr. J. Biotechnol., 4(6): 527-530.

Maita, M.; Shiomitsu, K. and Ikeda, Y. (1984). Health assessment by the climogram of hemochemical constituents in cultured yellow tail. Bull. Jap. Soc. Scient., 51: 205-211.

Masopust, J. (1998). Clinical biochemistry. Demanding and evaluation of biochemical investigation. Karolinum Praha. pp.832.

Mauel, M.J.; Miller, D.L. and Merrill, A.L. (2007). Hematologic and plasma biochemical values of healthy hybrid tilapia (*Oreochromis aureus* x *Oreochromis nilotica*) maintained in a recirculating system. J. Zoo Wildlife Med., 38(3): 420-424.

Maxine, M. and Benjamin, B.S. (1985). Outline of veterinary clinical pathology. 3rd edition, Colorado State Univ., Printed in India at Rekha printers PVT. LTD., New Delhi-110020.

McGowan, M.W.; Artiss, J.D.; Strandbergh, D.R. and Zork, B. (1983). A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clin. Chem., 29(3): 538-542.

Misaila, E.R.; Misaila, C.; Artenie, V. and Simalcsik, F. (2005). Effect of the chronic stress on some parameters of the metabolic-blood profile (MBP) of the farming Cyprinides. Fisheries and Aquaculture Development, XXX, HAKI, Hungary. pp.147-153.

Nicula, M.; Bura, M.; Simiz, E.; Banatean-Dunea, I.; Patruica, S.; Marcu, A.; Lunca, M. and Szelei, Z. (2010). Researches concerning reference values assessment of serum biochemical parameters in some fish species from *Acipenseridae*, *Cyprinidae*, *Esocidae* and *Salmonidae* family. Anim. Sci. Biotech., 43(1): 498-505.

Palanivelu, V.; Vijayavel, K.; Ezhilarasibalasubramanian, S. and Balasubramanian, M.P. (2005). Influence of insecticidal derivative (Cartap hydrochloride) from the marine polychaete on certain enzyme systems of the freshwater fish *Oreochromis mossambicus*. J. Environ. Biol., 26: 191-196.

Palikova, M.; Kopp, R.; Mares, J.; Navratil, S.; Kubicek, Z.; Chmelar, L.; Bandouchova, H. and Pikula, J. (2010). Selected haematological and biochemical indices of Nile tilapia (*Oreochromis niloticus*) reared in the environment with cyanobacterial water bloom. Acta Vet. Brno., 79: 63-71.

Qiu, T.; Xie, P.; Guo, L. and Zhang, D. (2009). Plasma biochemical responses of the plaktivorous filter-feeding silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) to prolonged toxic cyanobacterial blooms in natural water. Environ. Toxicol. Pharmacol., 27: 350-357.

Reitman, S. and Frankel, S.A. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28(1): 56-63.

Roche, H. and Boge, G. (1996). Fish blood parameters as a potential tool for identification of stress caused by environmental factors and chemical intoxication. Mar. Environ. Res., 41: 27-43.

SAS (Statistical Analysis System) (2006). SAS/STAT User's Guide, Version 9.1, SAS Institute Inc., Cary, NC, USA.

Shen, J. (2009). A one-stop reference source for comprehensive, up-to-date information on biology of silver, largescale silver and bighead carps and environmental risk assessment. Environ. Biol. Fish., 84: 341-344.

Svobodova, Z.; Vykusova, B.; Modra, H.; Jarkovsky, J. and Smutna, M. (2006). Haematological and biochemical profile of harvest-size carp during harvest and post-harvest storage. Aquacult. Res., 37: 959-965.

Tintos, A.; Miguez, J.M.; Mancera, J.M. and Soengas, J.L. (2006). Development of a microtitre plate indirect ELISA for measuring cortisol in teleosts and evaluation of stress responses in rainbow trout and gilthead sea bream. J. Fish Biol., 68: 251-263.

Trinder, P. (1969). Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Ann. Clin. Biochem., 6: 24-27.

Verma, S.R.; Rani, S. and Delela, R.C. (1981). Isolated and combined effects of pesticides on serum transaminases in *Mystus vittatus* (African catfish). Toxicol. Lett. 8: 67-71.

Wallaert, C. and Babin, P.J. (1994). Effects of temperature variations on dietary lipid absorption and plasma lipoprotein concentrations in trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol., 109(B): 473-487.

Weber, R.E. and Jensen, F.B. (1988). Functional adaptations in hemoglobins from ectothermic vertebrates. Ann. Rev. Physiol., 50: 161-179.

Wedemeyer, G.A.; Barton, B.A. and McLeay, D.J. (1990). Stress and acclimation. In: Methods for fish biology. Schreck, C.C. and Moyle, P.B. (eds). American Fisheries Society, Bethesda, MD. pp.415-489.

Yang, J.L. and Chen, H.C. (2003). Effects of gallium on common carp (*Cyprinus carpio*): Acute test, serum biochemistry, and erythrocyte morphology. Chemosphere, 53: 877-882.

Zhang, X.; Xie, P.; Wang, W.; Li, D. and Shi, Z. (2008). Plasma biochemical responses of the omnivorous crucian carp (*Carassius auratus*) to crude cyanobacterial extracts. Fish Physiol. Biochem., 34: 323-329.

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Experiential Teaching Method Applying in Career Planning Class for Associate Nursing Students in China

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Abstract: Objective Exploring an experiential teaching method in the Career Planning class for associate nursing students in China. **Methods** The random choosing of 100 nursing college students as a trial group (n=50) using experiential teaching method, and the selection of a control group (n=50) using the traditional teaching method. “Students’ satisfaction” and “Self-reported questionnaire” are used to evaluate the teaching effect. **Results** The application of experiential teaching in career planning helps students set up professional nursing values. Improved abilities in career planning、independent learning、logical thinking、communication skills and team cooperation are shown in the trial group (P<0.05). **Conclusion** The experiential teaching method applied in the career planning class is superior to the traditional teaching method. The experiential teaching method is a useful method of teaching, deserving popularization and further research.

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【**Keywords**】 Experiential Teaching; Career Planning Class; Associate Nursing Students

Introduction

Children in the United States have received career planning education since six years old. Chinese children do not receive career planning class until their university education. Furthermore not every child has such an opportunity, since only a few universities provide career planning classes. Such classes are 18 hours for 1 credit and taken as a junior or senior student. The content of career planning education in China include: employment orientation, how to collect employment information, maintaining a stable mental state, employment situation analysis, employment policies and regulations, resume and interview skills, how to start you own business, etc. The giving of lectures is supplemented by discussion groups and simulation interviews. Some universities invite enterprise personnel, alumni, and human resources experts to deliver thematic lectures as well. The reason for adopting the traditional lecture method is, firstly, a teacher delivers lectures on career planning theory on the stage while hundreds of students passively listen. And it is difficult to realize small-class-based lecture or individual counseling because of teachers shortage. Secondly, some difficulties, such as insufficient teaching conditions in China, security and safety issues including parental concerns, the disagreement of the public, values and cultural differences, etc, are met in the course of self exploration and career exploration activities in career planning class. Until now, most universities have not form career planning education system. In recent years, with the country place more value on college career development education, moreover many universities also strengthened the teaching materials. These publications go by titles on "employment guidance", "vocational guidance", "career planning", "career development", etc. However, most materials expound career planning knowledge and skills with theory, lacking giving attention to matters of practical implementation as case studies.

However, there are good examples in China. Some universities encourage students to organize monthly career development activities. For example, under the management of the Career Guidance Center, career development students carry out a great many monthly career development activities in the Shanghai Jiao Tong University, such as inviting professionals or career development teachers to deliver lectures, lectures by great professionals from different careers, community researches, visit diverse industries, professional seminars. Furthermore, some vocational guidance projects are carried out, such as employment counseling, interview workshops, professional competence development, etc. Compare to classroom teaching, Career development month activity and career development association activities are relatively rich. In addition, many colleges provide the opportunities for students to formulate career plans and business designs, when participate career planning competitions.

Career planning education in western countries started earlier than in China, mostly uses concept games and activities in the course of teaching and receives satisfying results. Because of the different cultural backgrounds, applying western career planning methods and content directly into Chinese education is not the way to proceed. However, the successful experiences of western countries set up a good example for China.

Based on experiential learning from the 1940s,

David· Kolb in 1984 put forth some proposals. Experiential teaching model is an integrative approach to teaching, as such actual experiment (doing), personal experience (sensing), observation (fact building), summarizing (thinking)". He revised his proposal in 2006. Experiential teaching could also be called " Discovery through the teaching", "Teaching through activity" or "interactive teaching". More specifically, students first take part in a series of activities to gain personal experience, and then they present and share ideas with each other under the teacher's guidance, and finally apply the understanding into daily life and work. Experiential teaching alters the passive and isolated study experience into introspection and shareable one, which activates thinking, make students fully participation. From the middle of the 90's, China rapidly commenced experiential teaching research. Although experiential teaching is widely researched in China, it is only on the level of theory, and lacks an adequate teaching plan. The limited application focuses on the teaching of sports, spoken foreign language, Chinese composition and reading, etc.

In view of the above limitations, this study aims to explore suitable experiential teaching in nursing career planning suitable to the current condition in education. This will not only inspire Chinese college students to develop a career planning consciousness, but also improve their career planning and management ability. Experiential teaching method in group is used in our career planning class, and the result is acceptable. Now introduce specific experience in report as follows.

1 METHODS

1.1 Sample

Selected first year nursing students of Zhengzhou university nursing college, were divided into two groups according to their orders of application. The trial group (n=50) used the experiential teaching method, and the control group (n=50) the traditional teaching method. All the students are from Henan province, 18 to 22 years old, with an age average of (20.45±0.31). There were no statistically significant differences in age, learning achievement and basic cultural knowledge ($P > 0.05$).

1.2 Implementation

1.2.1 The trial group implementation

The trial group applied experiential teaching methods. Perform in the second semester of the university, every two weeks for 2 academic hours (each hour included 50 minutes' teaching and 10 minutes' break), for a total of 18 hours. The textbook used was "Career planning for university students" authored by ZHONG GULAN and YANG KAI. The teaching content included self-exploration, career exploration, and then decision-making and setting of goals, Only then does one proceed of job hunting, and development of interview skills, etc. Below is how 2 academic hours of Career planning on "work world exploration" were conducted.

Step 1: preparation before classes Students were divided randomly into 10 groups of 5 each. A leader were elected for each group. Before class, each student drew a picture about "the work world in your eyes" based on their own experiences using colored pencils on white paper. It did not matter whether the pictures were beautiful or professional, but it did matter that they were drawn from one's own ideas. The teacher provided a book list and video on "What is nursing" and "Notes on being a nurse", to help nursing students prepare for effective hospital practice.

Step 2: actual experiment (doing) and personal experience (sensing)-hospital practice Students were arranged to visit the hospital as volunteers twice a week to work with the nurses. They interviewed one or two nurses about the work atmosphere, as well as the relationship among patients, doctors, nurses and other medical officers, the salaries, the satisfaction with daily tasks, the location, promotion, etc, In this way they became acquainted with the daily nursing work, communicated with the patients, became familiar with the hospital environment, gained an overall understanding and impression about nursing, all of which are important for the freshmen's future career planning and job choices. After social practice in the hospitals, they drew another picture about "the real work world in reality" to compare with the first picture they drew in Step 1. Furthermore, the students completed their personal on-site practice reports and nurse-interview reports. The teacher took photos and kept confidential record.

Step 3: group discussion and reflection In the first academic hour, 20 minutes were spent to group discussion about the hospital visit experience, feelings and pictures. After evaluation, analysis and discussion, each group assigned one student as the reporter to share with the whole class. To sum up, the main conclusions were "Working in the hospital as a nurse is time-consuming and energy-consuming", "Nurses is patient and busy", "and Nurses' salary is acceptable", etc. After discussion, several questions were brought out in the course of the work world exploration, such as "What are the methods of explore the work world", "How to interview nurses more effectively", "What kind of jobs can nursing students hunt after graduation", "What should the nursing students prepare for the future career" , etc. The presentation and discussion all over the class spent 25 minutes. At last, the teacher summed up the class in 5 minutes. The task of the teacher is to control the whole process in case discussion away from the topic.

Step 4: summary (thinking)-combining theory and practice In second academic hour, the teacher delivered a speech about how to explore the work world according the questions that the students put forward. It spent 20 minutes. The content of the speech include "why we should know more about future jobs", "what kind of information should we explore about the work world", "what the current situation of job market", "the opportunities and challenges in the competitive society","provide various category of jobs: full-time jobs, part-time jobs, SOHO(small office house office), establish a business, etc","the choices of further study","the skills and methods of career exploration" , "nurse-interview reports at best cover: what do you do daily work, how did you find this job, how you view the future trend of this job, is there any career planning in your work field, what requirements for being a excellent nurse, what do you like most and dislike most as far as your job, which part you are most satisfied and most unsatisfied, what kind of problems you met when enter into nursing career, is there any special suggestion for nursing students who are freshmen and who are about to hunt jobs, could you introduce several acquaintances to be my next interviewees, what else methods do you think could help me about nursing career". After that, apply "brain storm" make the students think about that what else jobs can nursing students hunt after graduation apart from nurses in the hospitals and nursing teachers in the college schools. And the results were healthcare nurses, massagists, nurse practitioners, clinical specialists, nurse midwives, nurse anesthetists, Psychiatrists, work for the Medicines Companies and Medicare offices, etc. It spent 10 minutes. And then, group discussion about "what did you do and what will you do about career exploration". It spent 10 minutes. Finally, group presentations cost 10 minutes. What most students have already done were overall evaluation of themselves and careers, information collection from publications, videos, and Websites, profession interviews, visiting work places and social practices. What most students would do next after discussion were formal and detailed evaluation (Holland career interest tests, career personalities tests, career value tests, listing professional skills, etc.), examples of parent-characters and relative-characters, Specialty Practices, broaden possible career choices, making full use of alumni resources, etc. The teacher summarized and reviewed the whole class, and encouraged students recording experiences and the work world exploration methods, so that put into practices.

Step 5: assignments outside the class and preparation for next class Students were asked to draw up the social practices and profession interviews plans of their own, and carry out regularly. Since the exploration of one self and the work world had been learned, the next lesson-"decision and action plans" should combine above two aspects. What each group should do was handing in a report about how to put what you learn into practice. Students should work with the other group members to complete the reports. The teacher marked the reports as a part of final examination score.

1.2.2 The control group implementation

The control group applied traditional teaching methods, which means the teacher standing on the platform, teaching theories with a few questions and answers, no groups for students. Perform in the second semester of the university, every two weeks 2 academic hours(each hour includes 50 minutes' teaching and 10 minutes' break), 18 hours in total, "Career planning for university students" as the textbooks(the authors are ZHONG GULAN and Yang Kai), teaching content are similar to the trial group.

2 RESULTS

After finish the whole lessons of career planning at the end of semester, each student of the two groups was asked to complete "The ability score of students' career planning" (Form 1) and "Self-reported questionnaire" (Form 2). The results of the trial group were superior to that of the control group, which demonstrated that experiential teaching method applying in career planning class is better than the traditional teaching method. And students in the trial group were more satisfied about the teaching effects.

Form 1. The comparison of the ability score of students' career planning (n=50, $\bar{x}$ ±s)

list	before		t	P	after		t	P
	the trial group	the control group			the trial group	the control group		
Self-recognize	3.37±0.05	3.48±0.05	-1.58	0.12	3.87±0.40	3.65±0.46	3.48	0.00
Career-recognize	3.32±0.06	3.34±0.06	-0.20	0.85	3.90±0.46	3.69±0.41	3.15	0.00
Career decision	2.90±0.06	2.99±0.07	-0.91	0.36	3.47±0.61	3.26±0.60	2.38	0.02
Planning-recognize	3.22±0.05	3.26±0.06	-0.48	0.63	3.89±0.49	3.65±0.45	3.41	0.00
Action adjustion	3.18±0.06	3.20±0.08	-0.21	0.84	3.79±0.56	3.44±0.59	4.04	0.00

Form 2. The comparison of the Self-reported questionnaire for teaching effect (n=50, $\bar{x} \pm s$)

list	the trial group	the control group	t	P
Study by oneself	8.89±1.25	8.25±1.13	3.40	0.00
Logical thinking	7.65±1.32	7.25±1.24	2.35	0.02
communication	8.90±1.21	8.35±1.23	3.39	0.00
Team-work	7.45±1.28	7.02±1.19	2.62	0.00

3. DISCUSSION

The application of experiential teaching in the career planning helps students set up professional nursing values. Better career planning ability, independent learning ability, logical thinking ability, communication skills and team cooperation ability are shown in trial group (P<0.05). Experiential teaching method is a useful method of teaching, deserving popularization and further research.

Nurses in China are busy with injection and delivering medication, without time educating the patients. The main reason is the shortage of nurses in China. Compared to the proportion of the number of nurses versus that of doctors 10 in America, there is less than 1 in China.

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Reference

1. Colin. Bierce Zhao, Huang RongHua translation experience in teaching power. [M]. Guangzhou: sun yes-men university press, 2003:23-46.
2. BAO HONGJIANG. Experience training - new way of human capital accumulation [D]. Chengdu, southwestern university of finance and economics, 2006:13-16.
3. LEXIAORONG. University of experiential teaching career planning design [D].shanghai: east China normal university, 2008:14-15.
4. DU XIAOXIA, Tian ZiRong, HanJie. Nurse self career planning an investigation into the actuality of the analysis and counter measures [J].diabetes care med, 2008,43 (2) : 131-185.
5. ZHONG GULAN, Yang opened. College career development and planning. 6th edition [M]. London: Comell university press, 2010:15-78.
6. YUAN YI, Geng GuiLing. Nursing comprehensive experimental teaching of clinical situation drill construct simulation of students' subjective practice [J]. J nurse education magazine, 2007,22 (21) : 1940.
7. GAO XIU. Medical PBL teaching model class create atmosphere [D]. Beijing: China medical university, 2010:12 2007,22（2）：40.

MicroRNA differential expression profile in cholangiocarcinoma cell line and normal bile duct cell line

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Abstract: The aim of this study was to explore microRNA differential expression profile between cholangiocarcinoma cell line QBC939 and normal bile duct cell line, for further studies on functions of microRNA in pathogenesis of the cholangiocarcinoma. Six samples from QBC939 cell line and another 6 samples from normal bile duct cell line were chosen as the experimental group and the control group, respectively. MiroRNA profiles in these samples were analyzed by microarray. The threshold value used to screen up and down regulated microRNA were Fold Chang>2. Six microRNAs were found up-regulated in the experimental group were more than 8 times compared with control group. In addition, 5 microRNAs were found down-regulated 8 times compared with control group. These differential expressions of miroRNA might be related to the formation and metastasis of cholangiocarcinoma.

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Keywords: cholangiocarcinoma; MicroRNA; cell line; metastasis

1. Introduction

MicroRNA is a kind of conservative non-coding RNA,with a length of 19 to 25 nucleotide. It is widely distributeed in virus, plant and higher mammal. The research shows that microRNA plays the key role in controlling development, apoptosis, multiplication and differentiation of the cells. Due to the close relations between its unbalance expression and the tumor, the relations between microRNA and malignant tumor become a hot spot[1-3]. Cholangiocarcinoma is a kind of malignant tumor derived from bile duct epithelium in or out of the liver. In recent years, along with the development of imaging diagnostic technology, the detection rate of cholangiocarcinoma increases. It is reported that in the Primary Tumors of Liver, cholangiocarcinoma ranks only second to liver cancer. As the formation and development of cholangiocarcinoma is a complex process with various steps and factors, the pathogenesis is still not so clear. The correct diagnosis in early stage and timely reasonable treatment has important significance to improve the long-term survival rate for the cholangiocarcinoma patient. However, its clinical manifestation is not so typical that it brings great difficulty to early diagnosis and treatment, and it is often the case that the condition has been in the late fall when patients are with obvious clinical symptoms. The proposing of microRNA provides a new concept to the pathogenesis, early diagnosis and targeted treatment [4, 5]. Applying microRNA chip technology to screen the differential expressed microRNAs from bile duct cancer cell strains QBC939 and normal bile duct epithelia cell strains, this research provides important theoretical foundation for further study in the forming mechanism and effective treatment means of cholangiocarcinoma.

2. Material and Methods

The materials are purchased from the following company: cholangiocarcinoma cell line QBC939 and normal bile duct epithelia cell line are all from CAMS(Chinese Academy of Medical Sciences), McCoy'5A medium, high glucose DMEM medium, and super and standard fetal calf serum are from American HyClone Company, Peniciline /Streptomycin liquor and trypsin are from GIBCO company, cell RNA total extraction reagent Trizol is from American Invitrogen company, MiRCURY ^{TM} chip is from Denmark Exiqon company, Axon GenePix 4000B hydrone RNA chip scanistor is from American Melecular Devices company, PCR thermal cycler is from Japanese Takara Bioengineering Co. Ltd, and RT-PCR primer is composed by Life Technologies.

Cholangiocarcinoma cell line QBC939 and normal bile duct epithelia cell line are cultivated in the 37℃ 5 % CO2 incubator with 10% fetal calf serum McCoy'5A medium and 10% super fetal calf serum medium, respectively. Six samples from QBC939 cell line and another 6 samples from normal bile duct cell line were chosen as the experimental group and the control group, respectively, and

numbered as Q-1、Q-2、Q-3、Q-4、Q-5、Q-6 and H-1、H-2、H-3、H-4、H-5、H-6。

Use Trizol to extract total RNA of the 12 cell sample which are in the logarithmic growth phase, then determine the absorbance value D(260) 、 D(280)and D(230) with ultraviolet spectrophotometer, and then quantify the total RNA while calculate OD260/280 value and OD260/230 value, combing formaldehyde degeneration gel electrophoresis to analyze the quality of RNA.

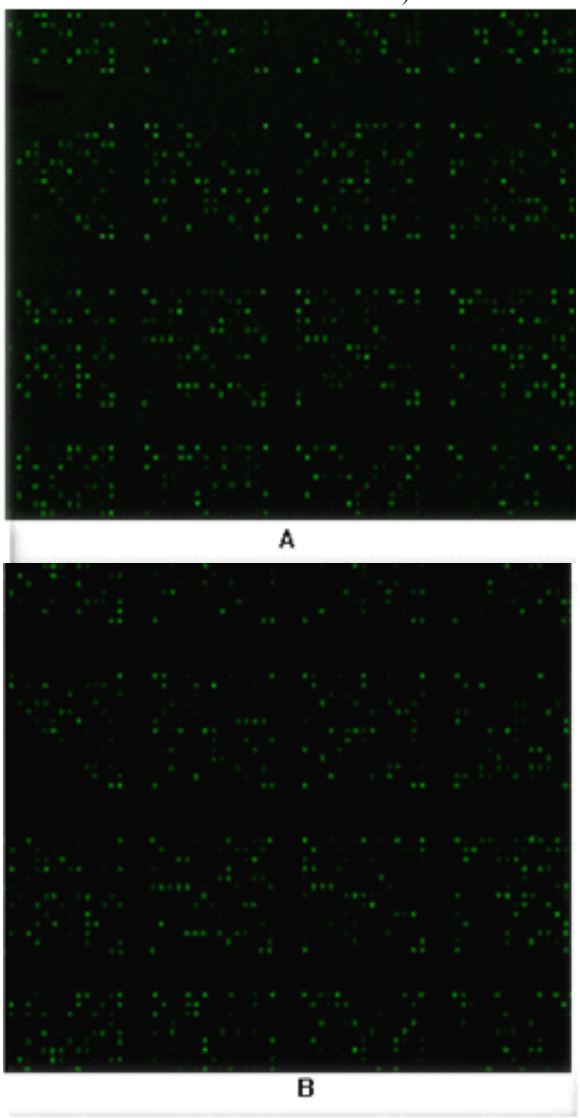
Take 5 μg of total RNA from the 12 samples to prepare fluorescently tagged probe, use miRCURY LNA™ to mark kit and fluorescently tagged microRNA using marker enzyme with Hy3™/Hy5™, obtaining the fluorescent light so as to hybridize the chip. Under standard conditions, using PhalanxTM thermal contraction hybridizing bag, hybridized the marked probe and miRCURY™ chip (with each sample detected 4 times by the chip). After hybridized, take out the chip and wash it, scanning it immediately while the chip is dry, and then the fluorescence intensity of the chip is scanned. Then mating software is applied to do the analysis of image and data, and the differential expressed gene ($P<0.05$) is screened out, and lastly, cluster analysis to these differential genes is done.

MicroRNA Chip data employs GenePix Pro6.0 software to design the t-test grouply according to fluorescence intensity after the extraction of the original data, and P<0.05 has statistic meaning. The PCR data adopts 2 - △△ CT to do the analysis and determines up-regulation, down-regulation and range of the molecular to be examined according to whether 2 - △△ CT is greater or less than zero.

3. Results

In order to detect whether the total RNA of the 12 samples can meet the following demand of the chip experiment, the OD260/280 value and OD260/230 value are determined by ultraviolet spectrophotometer and the total RNA quality is analyzed through observing the electrophoretogram. The results detected by ultraviolet spectrophotometer are as follows: the OD260/280 values of the total 12 samples of Q-1、2、3、4、5、6 and H-1、2、3、4、5、6 are 2.02、2.08、2.04、2.03、2.09、2.02、2.02、2.03、2.05、2.07、2.03、2.09, respectively, which are all between 1.8 and 2.1. The result shows that there is no degradation, protein and DNA pollution. The OD260/230 value of the 12 samples are 2.15、2.18、2.09、2.13、2.12、2.21、2.12、2.15、2.19、2.10、2.18、2.14, respectively, which are all above 2.0. The result shows that there is no pollution of organic solvent. The electrophoresis result shows that the total RNA of two groups of sample all have clear 18S and 28S strip, and the quality of the sample preservation are good. All of the results show that the total RNA quality fit the following microRNA demand of chip experiment.

The hybridized chip is scanned by GenePix 4000B (Picture 1). Compared to the normal bile duct epithelia cell line, the obviously differential expression of microRNA up-regulated or down-regulated two times of cholangiocarcinoma cell line totals 133, among them, 72 microRNA are above 2 times, 6 microRNA are above 8 times(Table 1); and 61 microRNA are below 2 times,5 microRNA are below 8 times with $P<0.05$（Table 2).



Picture 1 part of the chip scanning picture of sample microRNA (A represents the Q-1microRNA chip scanning picture of cholangiocarcinoma cell line, while B represents H-1microRNA chip scanning picture of normal cell line)

Table 1. MicroRNA with the ratio of the differential expression between cholangiocarcinoma cell line QBC939/ normal bile duct epithelia cell line above 8 times

microRNA with expression up-regulated 8 times		
Name	times	P
hsa-miR-21	91.7	*0.003*
hsa-miR-141	76.9	*0.002*
hsa-let-200b	48.5	*<0.001*
hsa-miR-15a	24.4	*0.017*
has-miR-23a	21.6	*<0.001*
hsa-miR-15b	10.8	*0.006*

Table 2. MicroRNA with the ratio of the differential expression between normal bile duct epithelia cell line / cholangiocarcinoma cell line QBC939 above 8 times

microRNA with expression down-regulated 8 times		
Name	times	P
hsa-miR-125a	89.6	*<0.001*
hsa-miR-31	85.2	*<0.001*
hsa-let-7b	49.1	*<0.001*
hsa-miR-95	24.8	*<0.001*
has-miR-320	17.9	*<0.001*

4. Discussions

MicroRNA is widely existed in various kinds of creatures. There are about 1,000 kinds of microRNAs in the human body at present; they regulate most protein-coded gene and a part of non-protein-coded gene. It is estimated that microRNA takes up about 3% human being's gene in the way of controlling the expression of mRNA by multiple microRNA corresponding to one mRNA[6, 7]. These microRNAs inhibit the expression of targeted gene through inducing the incision and degradation of mRNA, translated inhibition or other forms of regulatory mechanism[8]. It has been proven that microRNA plays tremendous regulatory role in the formation of various tumors[9-11]. According to its different roles, these microRNAs can be divided into carcinogenicity microRNAs and anti- carcinogenicity microRNAs. The cancer gene may be promoted by the up-regulated microRNAs in the tumor tissues, while inhibited by the down-regulated ones. Calin found that microRNA-15a and microRNA-16a have down-regulated or lost expression in most chronic lymphocytic leukemia patient. Soon afterwards microRNA was found disorderly expressed in lung cancer, thyroid caner, breast cancer, gastrointestinal cancer, liver cancer and so on successively. At present, these microRNAs have been classified into oncogene or anti-oncogene according to their role in the tumor. Through appling the microRNA chip to analyze the differential expression profiling of microRNA between cholangiocarcinoma cell line QBC939 and normal bile duct epithelia cell line, this research screens out 6 microRNA which have differential expression up-regulated 8 times in the cholangiocarcinoma cell lines, they are microRNA-200b , microRNA-21 , microRNA-141 , microRNA-15a , microRNA-23a and microRNA-15b respectively ; 5 microRNAs which have differential expression downregulated 8 times, and they are microRNA-125a , microRNA-31 , microRNA-95 , microRNA-320 and let-7b, respectively. The result shows that the imbalanced expression of these microRNAs has closely relationship with the growth, apoptosis and invasion of the cholangiocarcinoma cell lines.

The family of microRNA-200 includes the member of microRNA-200a , microRNA-200b , microRNA-200c, microRNA-141, microRNA-429 and so on. The research has been shown that microRNA-200 family may enhance the expression of E- calcium-adhere protein through inhibit the expression of ZEB1 、 ZEB2 so as to play the important part in the process of transferring epithelial cell and mesenchyme. This research finds that in the cholangiocarcinoma cells, there are expressions of microRNA-200b and microRNA-141 in the microRNA-200 family. The target gene of microRNA-200b is PTPN12, which can inhibit the cancer through dephosphorylating to c-Ab1 and Src. The target gene of microRNA-141 is CLOCK, which not only plays important role in regulating the biorhythm of the organisms, but also inhibits cell division and promotes cell apoptosis as anti-oncogene, so we can consider that microRNA-141 promotes the proliferation of cholangiocarcinoma cells through inhibiting the expression of CLOCK. MicroRNA-21 has unusual expression in various malignant tumors[12], and most anti-oncogenes are the direct target genes of microRNA-21, such as TPM1 , PDCD4 and maspin. The inhibition to the expression of anti-oncogenes not only benefit the growth of the tumor cells, but also plays important role to the invasion and transference of tumor. This research finds that microRNA-21 has obviously high expression in cholangiocarcinoma cell lines, which shows that microRNA-21 and cholangiocarcinoma have close relations. In addition, microRNA-23a 、 microRNA-15b 、 microRNA-125a , microRNA-

31，microRNA-95，microRNA-320 and let-7b have obviously differential expression between cholangiocarcinoma cell lines and normal bile duct epithelia cell line, which hints that they also play important role in the formation and development of cholangiocarcinoma, and their role needs to be further studied.

Appling microRNA chip technology, this research sets up the expression chart of microRNA in cholangiocarcinoma, and the differentiate microRNA screened out may be used as the biology marker of screening, diagnosing and prognosis monitoring of cholangiocarcinoma. However, the present research is still in the initial stage so that new microRNAs related with cholangiocarcinoma needs to be further searched. At the meantime, the functions of all kinds of microRNAs also need to be further explored.

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References

1. Blitzblau RC, Weidhaas JB. MicroRNA binding-site polymorphisms as potential biomarkers of cancer risk. Mol Diagn Ther, 2010, 14(6): 335-342.
2. Nana-Sinkam SP, Croce CM. MicroRNA dysregulation in cancer: opportunities for the development of microRNA-based drugs. Idrugs, 2010, 13(12): 843-846.
3. Shah AA, Meese E, Blin N. Profiling of regulatory microRNA transcriptomes in various biological processes: a review. J Appl Genet, 2010, 51(4): 501-507.
4. Allen KE, Weiss GJ. Resistance may not be futile: microRNA biomarkers for chemoresistance and potential therapeutics. Mol Cancer Ther, 2010, 9(12): 3126-3136.
5. Jin H, Tuo W, Lian H, Liu Q, Zhu XQ, Gao H. Strategies to identify microRNA targets: new advances. N Biotechnol, 2010, 27(6): 734-738.
6. Huang K, Zhang JX, Han L, You YP, Jiang T, Pu PY, Kang CS. MicroRNA roles in beta-catenin pathway. Mol Cancer, 9: 252.
7. Tessel MA, Krett NL, Rosen ST. Steroid receptor and microRNA regulation in cancer. Curr Opin Oncol, 2010, 22(6): 592-597.
8. Iguchi H, Kosaka N, Ochiya T. Versatile applications of microRNA in anti-cancer drug discovery: from therapeutics to biomarkers. Curr Drug Discov Technol, 2010, 7(2): 95-105.
9. Lin PY, Yu SL, Yang PC. MicroRNA in lung cancer. Br J Cancer, 2010, 103(8): 1144-1118.
10. Wu WK, Lee CW, Cho CH, Fan D, Wu K, Yu J, Sung JJ. MicroRNA dysregulation in gastric cancer: a new player enters the game. Oncogene, 2010, 29(43): 5761-5771.
11. Wang J, Wang Q, Liu H, Hu B, Zhou W, Cheng Y. MicroRNA expression and its implication for the diagnosis and therapeutic strategies of gastric cancer. Cancer Lett, 2010, 297(2): 137-143.
12. Pan X, Wang ZX, Wang R. MicroRNA-21: a novel therapeutic target in human cancer. Cancer Biol Ther, 2010, 10(12): 1224-1232.

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The Application of Fuzzy Modeling to Hazard Assessment for Reinforced Concrete Building Structures Due to Pipeline Failure

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Abstract: In this article, the application of fuzzy modeling to hazard assessment for reinforced concrete building structures due to pipeline failure was implemented. Damage assessment due to sewer pipeline failure is a very important issue in urban regions in Egypt. By combining ground deformation patterns, well-known damage category criteria, the potential damage of adjacent buildings can be assessed due to different parameters of pipeline deterioration. In this study, the well-known computer program ANSYS with geotechnical module "CivilFEM" is used considering nonlinear elastic soil behavior. The finite element model is chosen to investigate the influence of four different parameters of pipeline deterioration at the same time such as pipeline settlement, settlement location, building location with respect to pipeline and burial depth on the building damage category. The results were implemented in a fuzzy based assessment system for reinforced concrete building structures to evaluate the damage category of building. A criterion to define membership functions for each parameter, as input to the fuzzy engine, as well as the rule base was described. The fuzzy output as damage category was briefly validated by using numerous examples for different values that was chosen randomly to cover the whole range of 4 parameters to get the results first in fuzzy system, then running the same values using ANSYS and results were consistent in the two methods. Fuzzy logic support system showed to be a powerful tool in forecasting potential damage in buildings due to the association of different parameters in pipeline deterioration.
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Keywords: Damage category, pipeline failure, membership functions, rule base and fuzzy assessment system.

1. Introduction

In a major developing country like Egypt the problem of sewer pipeline deterioration draws much attention. Therefore, the influence of pipeline failure on adjacent structures is very important to investigate.

A finite element computer program "ANSYS+CivilFEM"[1], was employed to perform the analysis and investigate the general failure mechanisms of soil- structure interaction. This analysis produced a large amount of output data[7]. The pipeline failure can induce vertical settlement of the foundation of the adjacent structure, which results in noticeable damage of buildings. The report by Aye [2] was used as a basic reference in ground deformation prediction and building damage assessment. For cut-and-cover excavation zone, the work of **Peck** [3], **Clough** [4] was used whereas published papers of **Burland** [5], **Boscardin and Cording** [6] were applied for bored tunnels. Also, **Metwally** [7] has evaluated the damage assessment of building due to deterioration of pipelines. This was the base of our previous work by Emarah *et al* [18] that was extended in this research. The damage categories are based directly on the descriptions of damage provided in Table 1.

The calculation of damage category by "ANSYS+CivilFEM" software is time consuming and it doesn't cover the entire operation range. Therefore, an expert system will be implemented to predict the degree of damage for different parameters of pipeline failure.

One of the most important applications of expert systems in engineering is fuzzy logic. The fuzzy set theory was developed by **Lofty Zadeh** [8] in 1965 to deal with imprecise and uncertain phenomena often presented in real-world applications. It provides[9]a powerful tool to model uncertainty associated with lack of information.

Consequently, fuzzy logic provides an efficient way of handling the uncertainty for complex systems without sufficient data or only with vague information [10,11]. The fuzzy controller has been used [12] for optimization of the active control of civil engineering structures [13-17]. The main advantages of the fuzzy controller are [14]:

- It is one of the few mathematical model free approaches to system identification and control which makes the system easier to design than developing an accurate mathematical model of the structural system needed for control system design. This can be done by using human experience and expertise to implement the fuzzy controller.
- The fuzzy controller can be adaptive by modifying its rules or membership functions and employing learning techniques.

In this study, a fuzzy rule-based decision support system is developed to determine the damage category of a building for a wide range of different parameters, depending on differential settlement underneath the building crack width and number of cracks obtained from ANSYS model. This was accomplished for two different parameters [18] and will be extended in this study for four parameters.

Table 1 Building damage classification after Burland [5] and Boscarding and Cording [6].

Risk Category	Degree of Damage	Description of Typical Damage	Approximate Crack Width (mm)
0	Negligible	Hairline cracks	Null
1	Very Slight	Fine cracks easily treated during normal decoration	0.1 to 1
2	Slight	Cracks easily filled. Several slight fractures inside building. Exterior cracks visible	1 to 5
3	Moderate	Cracks may require cutting out and patching. Door and windows sticking	5 to 15 or a number of cracks > 3
4	Severe	Extensive repair involving removal and replacement of walls, especially over doors and windows. Windows and door frames distorted. Floor slopes noticeably.	15 to 25 but also depends on number of cracks
5	Very Severe	Major repair required involving partial or complete reconstruction. Danger of instability.	> 25 but depends on number of cracks

2. Fuzzy inference systems

Fuzzy logic [9] is a kind of multi-valued logic utilizing fuzzy sets to perform approximate reasoning. Additionally, a fuzzy rule-based system is a methodology for the interpretation of natural language, which is essential for linguistic expressions. Fuzzy rules and fuzzy reasoning are the fundamentals of fuzzy inference processes that are utilized to derive meaningful conclusions from ambiguous information [11].

In this context, Fuzzy Inference Systems (FIS), also known as fuzzy rule-based systems, are well-known tools for the simulation of nonlinear behaviors with the help of fuzzy logic and linguistic fuzzy rules. There are currently several popular inference techniques developed for fuzzy systems, such as **Mamdani and Assilian** [19], and **Takagi and Sugeno** [20]. Mamdani FIS was selected to be used in this study. In the Mamdani FIS, inputs and outputs are represented by fuzzy relational equations in a canonical rule based form. These linguistic IF-THEN rules are associated with logical connectives, namely AND, OR, ELSE.

Another important point that should be explained about fuzzy rule-based systems is how the aggregation of fuzzy rules is performed. It is necessary to obtain an overall conclusion through a consideration of results from each rule. The combination of entire outcomes in a rule-base is referred as the aggregation of fuzzy rules. Similar to the association of fuzzy variables, there are two cases used in the aggregation process, namely conjunctive and disjunctive systems of rules [10-11]. A graphical representation of a Mamdani inference system with two rules and two crisp inputs is shown in Figure 1.But it is necessary to obtain a single value instead of a region to reach a decision; therefore, the solution should be defuzzified to get a crisp outcome. The centroid defuzzification method was chosen in this research.

3. Description of basic model

Figures 2 and 3 depict the full three-dimensional geometry model which was used to quantify the interaction between sewer pipeline and the reinforced concrete building with masonry in-fill walls in the coupled analysis. The assumed values in this parametric study are deduced from the practical observations of the deteriorated sewer pipes within the Greater Cairo sewer network[7]. The pipeline comprises 20 pipe segments, where the connections between them are contact element. The type of contact element of pipes connection was taken as “no separation contact element”. In this “no separation

contact" element, the two contact surfaces "target and contact surfaces" are tied, although sliding is permitted. The pipeline is encased in a homogeneous, continuous, and isotropic soil mass. In addition, frictional slip is allowed between pipe and soil. The used data are shown in Table 2. The column's spacing of building in the two directions s = 5.0 m, and height of each level h = 3.0 m. The properties of structural materials taken for deformation and failure prediction calculations are shown in Table 3. The contact element between the foundation of the building and the soil was taken rough element. In this element (rough contact), the two contact surfaces (target and contact surfaces) are not slipping, although separation is permitted.

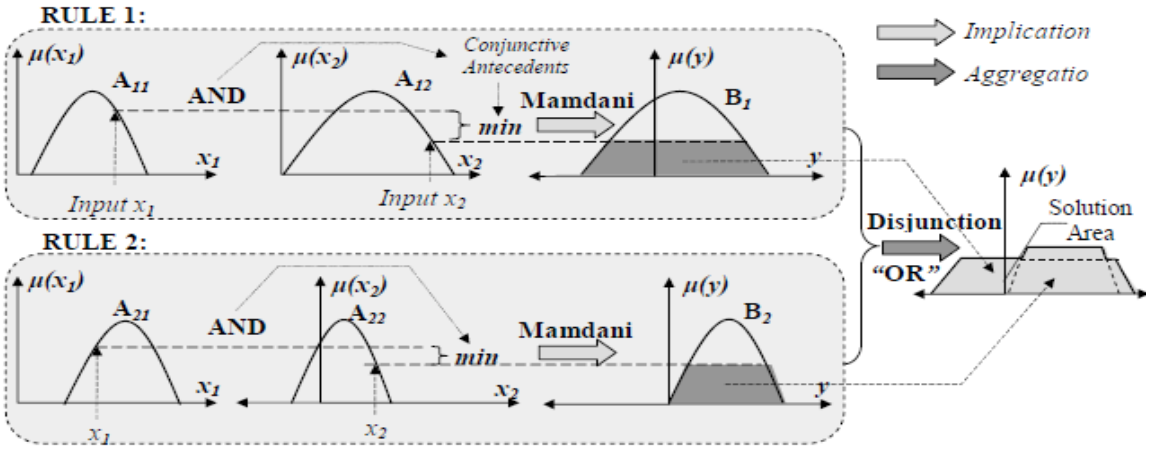

Figure 1 Graphical illustration of Mamdani inference methodology (2 rules and 2 inputs) [9].

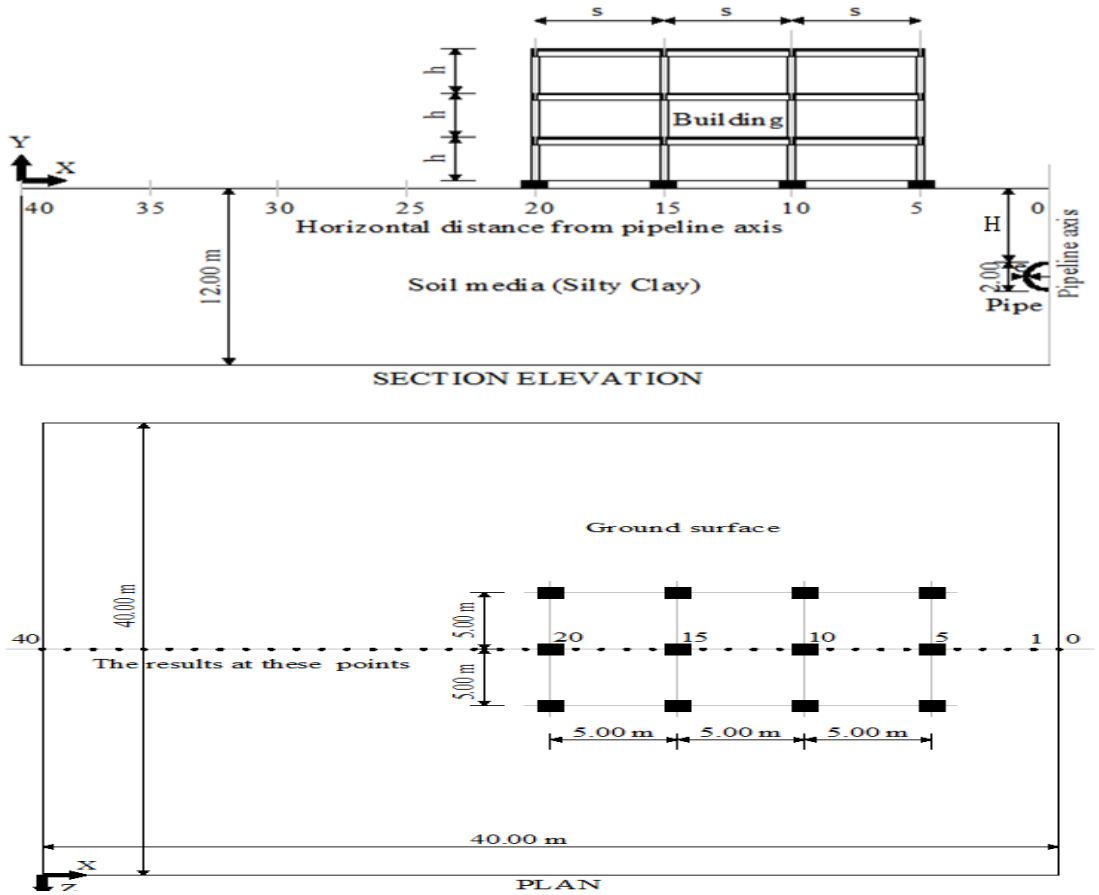

Figure 2 Geometric model.[18]

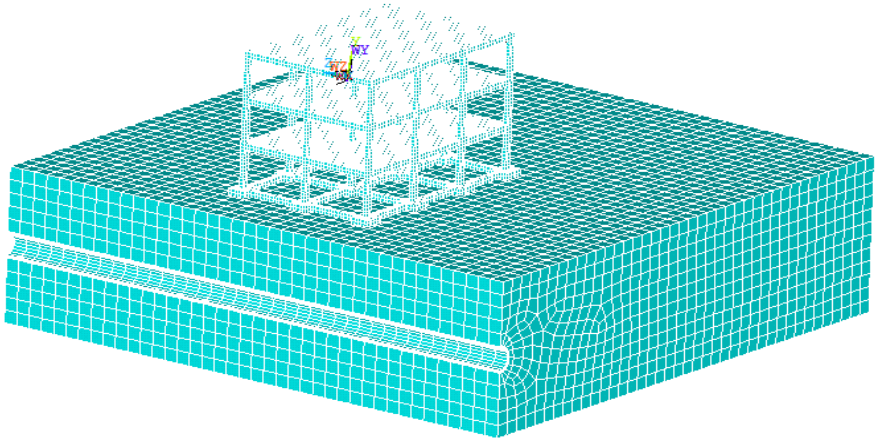

Figure 3 FEM model.[18]

Table 2 Soil and pipeline properties [7].

Soil properties		Pipeline properties	
Soil elastic modulus E_s	2000 t/m^2	Pipe diameter D (interior)	2.00 m
Soil Poisson's ratio υ	0.35	Wall thickness of concrete e	0.20 m
Soil cohesion C	2.00 t/m^2	Pipe length Lp	2.00 m
Angle of internal friction ϕ	30^o	Number of pipes in pipeline	20 pipes
Density of soil over pipe γ	1.85 t/m^3	Concrete elastic modulus E_c	3.5E6 t/m^2
Soil height above crown H_t	5.0 m	Concrete Poisson's ratio υ_c	0.20
μ (Between soil& pipes)	0.32	μ (Between pipes segments)	0.60

Table 3 Structural material data [7].

Properties	Notation & Unit	Building elements
Density	γ (t/m^3)	2.5
Compressive stress*	f_c (kg/cm^2)	90
Tensile stress*	ft (kg/cm^2)	10.8
Shear stress*	q (kg/cm^2)	19
Young's modulus	E (t/m^2)	2.1E06
Poisson's ratio	N	0.20
compressive strain*	ε_c	0.003
tensile strain*	ε_t	0.003
Shear strain*	ε_s	0.003

*Allowable stress or strain

4. Inputs of fuzzy logic

The damaging impact of pipeline settlement on building performance has been shown to be a major problem for urban areas due to high reconstruction and maintenance costs. The assumptions of parametric study of this part are deduced from the practical observations of the deteriorated sewer pipes within the Greater Cairo sewer network [7].

4.1 Effect of pipeline settlement on building

The influence of settlement in the pipelines is explained by considering three values of vertical settlement in the middle six pipe segments; 1% D, 3% D, and 5% D, where D is the pipe diameter as shown in Fig. 4. Figure 5 shows the relation between the vertical settlement of building and the pipeline settlement. It is apparent that increasing the vertical settlement of pipeline leads to the increase of the deformations of the adjacent building.

Table 4 illustrates the results for evaluating the potential damage category for building due to different values of pipeline settlement. The results presented in this table show the values of differential settlement, tilting angle α for the base of building and illustrate the influence of pipeline settlement on the value of crack width. We can find out that, the maximum building deformation and damage at the maximum pipeline settlement. It is clear that the value of pipeline settlement plays an important role in building deformation and damage.

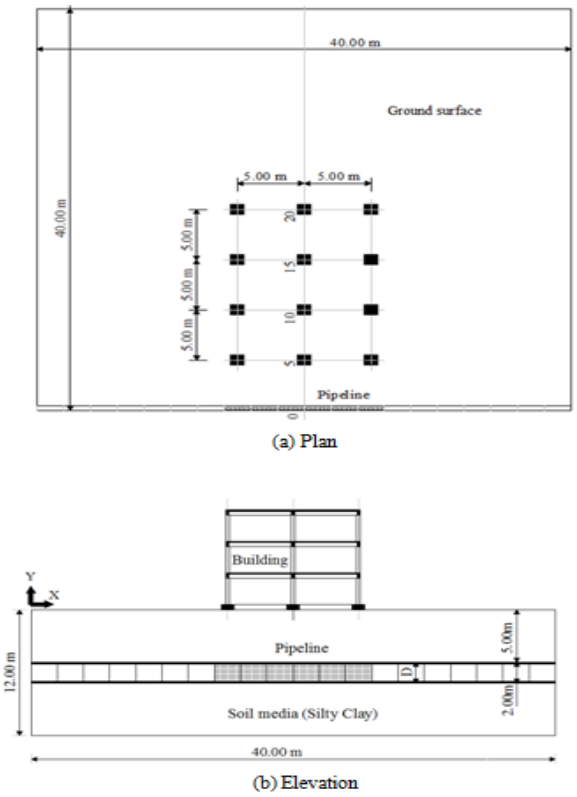

Figure 4 Location of pipeline settlement.[18]

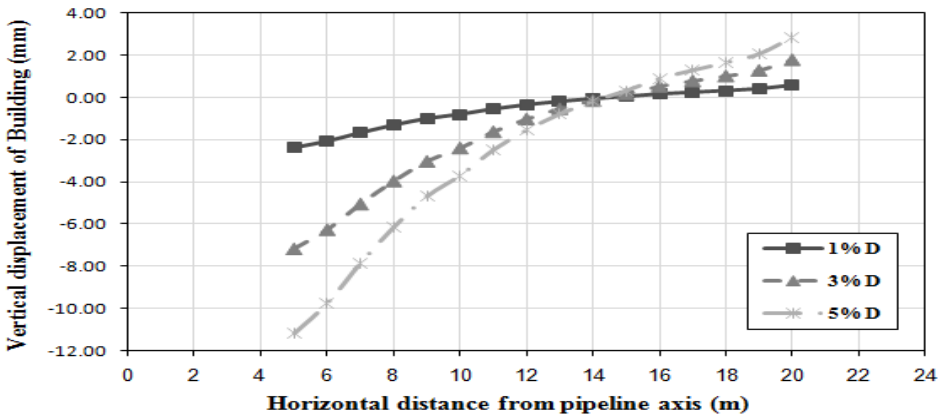

Figure 5 Effect of pipeline settlement on vertical settlement of building.[18]

Table 4 Evaluation of potential damage in building due to pipeline settlement.[18]

Properties	Case		
	1% D	3% D	5% D
Differential Sett.(ΔS)mm	2.94	8.93	14.04
Angle of Tilt (α) rad.	0.00020	0.00060	0.00094
Cumulative Maximum Tensile Crack Width (C_t) mm	0.88	2.80	4.79
Cumulative Maximum Principal Crack Width (C_p) mm	0.81	2.50	4.06
Damage Category	Very Slight	Slight	Moderate

4.2 Effect of settlement location on building

The influence of settlement location relative to the building in the pipelines is explained by considering three different horizontal locations of settlements as shown in Fig. 6. At case 1, the centerline of six pipe segments at the centerline of building (X =0.00m). At case 2, the start of six pipe segments at X=6.00m from centerline of building. At case 3, the start of six pipe segments at X=12.00m from centerline of building. The pipeline settlement value was taken 5% D where D is pipe diameter.

The influence of the settlement location on the vertical settlement of building is shown in Fig. 7. As seen, the vertical settlement of building decreases with increasing the distance to the location of pipeline settlement.

Table 5 illustrates the results for evaluating the potential damage category for building due to the location of settlement in the pipeline. The results presented in this table show the values of differential settlement, tilting angle α for the base of building, and illustrate the influence of pipeline settlement location on the value of crack width. We can find out that, the maximum results of building deformation and damage are for the nearest location of pipeline settlement (X=0.00m).

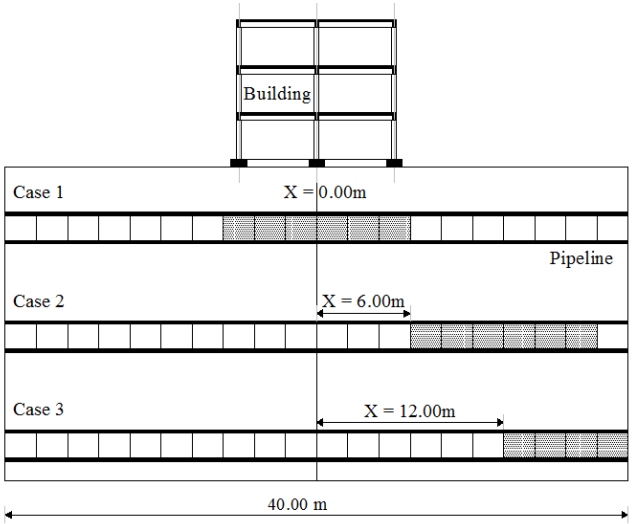

Figure 6 Location of vertical settlement of pipeline.

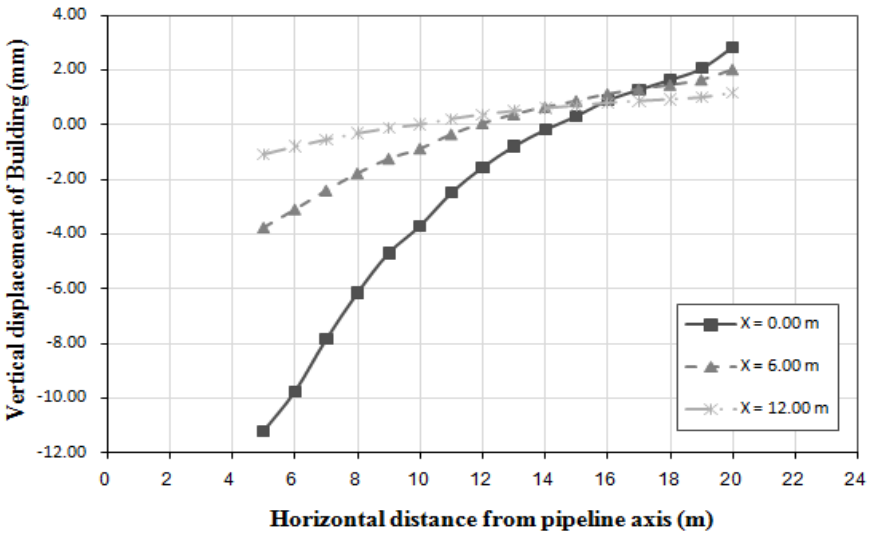

Figure 7 Effect of pipeline settlement location on vertical settlement of building.

Table 5 Evaluation of potential damage in building due to the location of pipeline settlement.

Properties	Case		
	X =0.00m	X =6.00m	X =12.00m
Differential Sett.(ΔS)mm	14.04	5.79	2.23
Angle of Tilt (α) rad.	0.00094	0.00039	0.00015
Cumulative Maximum Tensile Crack Width (C_t) mm	4.79	1.39	0.56
Cumulative Maximum principal Crack Width (C_p) mm	4.06	1.37	0.52
Damage Category	Moderate	Slight	Very Slight

4.3 Effect of burial depth on building

The influence of burial depth is demonstrated by considering three heights of soil above the crown of the pipe; 3, 5, and 7 m. Tables 2 and 3 give the properties of silty clay soil, pipe, and building respectively. The settlement value was fixed as 5% D (D is pipe diameter) in the middle 6 pipe segments. Figure 8 illustrates the influence of burial depth and pipeline settlement on the vertical settlement of building. We can notice that increasing the height of soil above the pipe decreases the building deformations.

Table 6 illustrates the results for evaluating the potential damage category for building due to settlement in pipeline and different burial depth. The results presented in this table show the values of differential settlement, tilting angle α for the base of building and illustrate the influence of different burial depth with settlement in pipeline on the value of crack width. We can find out that, the building damage is increasing by decreasing in the soil height above pipeline.

4.4 Effect of building location on building

The influence of building location relative to pipeline settlement is demonstrated by considering three different locations from the nearest side of building relative to the centerline of the pipeline (XB); 3, 5, and 7 m as shown in Fig. 9. The settlement value was taken 5% D (D is pipe diameter) in six pipe segments at (X=0.00m) as shown in Fig. 6. In case 1 the tensile and principal crakes are calculated at the first bay from (3m to 8m). In case 3 the tensile and principal crakes are calculated at the first bay from (7m to 12m).

The influence of the building location and pipeline settlement on the vertical settlement of building is shown in Fig. 10. As seen, the maximum numerical results obtained from the position of the nearest location (XB=3.00m) of the building to the pipeline.

Table 7 illustrates the results for evaluating the potential damage category for building due to building location and pipeline settlement. The results presented in this table show the values of differential settlement, tilting angle α for the base of building, significant difference for building damage for the all building locations relative to the pipeline settlement.

5. Damage evaluation of building using fuzzy logic tool

One of the most important applications of fuzzy logic is that it can be used for decision process based on available data and knowledge. This study aims to construct a decision support system for damage category of reinforced concrete building structures based on numerical solutions obtained from ANSYS results for a wide range of parameters. Four different variables that have influence on building damage were used as inputs for fuzzy system. Then a procedure using the fuzzy inference methodology was developed to determine the output of a fuzzy system. The global structure of FIS component is depicted in Fig. 11. The shape of membership functions is chosen by trial and error to get the best representation of each input and output parameters.

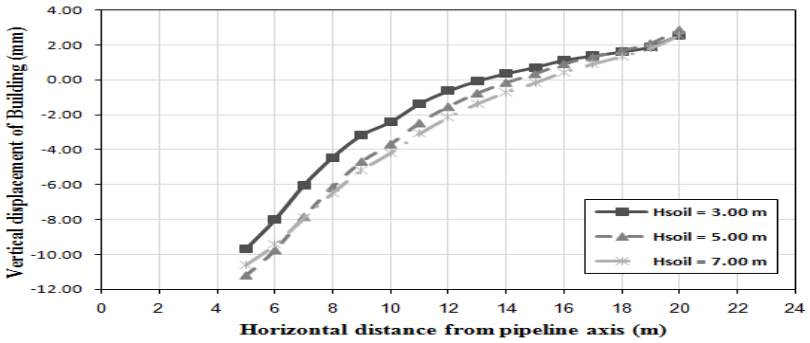

Figure 8 Effect of burial depth on vertical settlement of building. [18]

Table 6 Evaluation of potential damage in building due to the burial depth value.[18]

Properties	Case		
	H_{soil} = 3m	H_{soil} = 5m	H_{soil} = 7m
Differential Sett.(ΔS)mm	12.25	14.04	13.14
Angle of Tilt (α) rad.	0.00082	0.00094	0.00088
Cumulative Maximum Tensile Crack Width (C_t) mm	5.98	4.79	2.82
Cumulative Maximum Principal Crack Width (C_p) mm	4.55	4.06	2.92
Damage Category	Moderate	Moderate	Slight

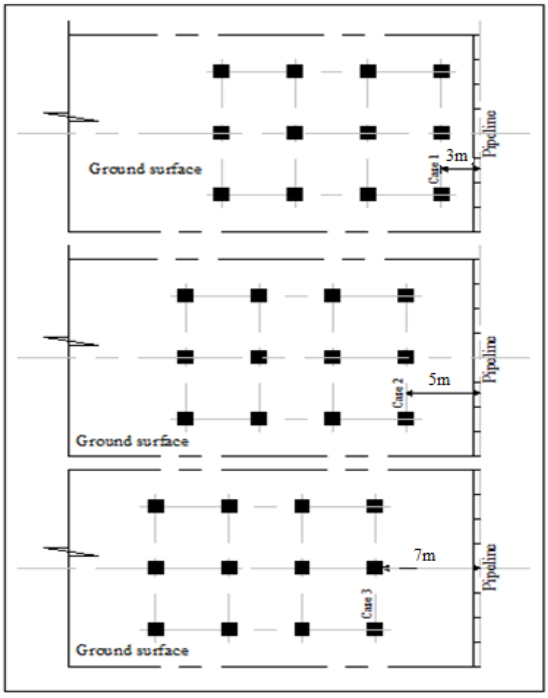

Figure 9 Different building location.

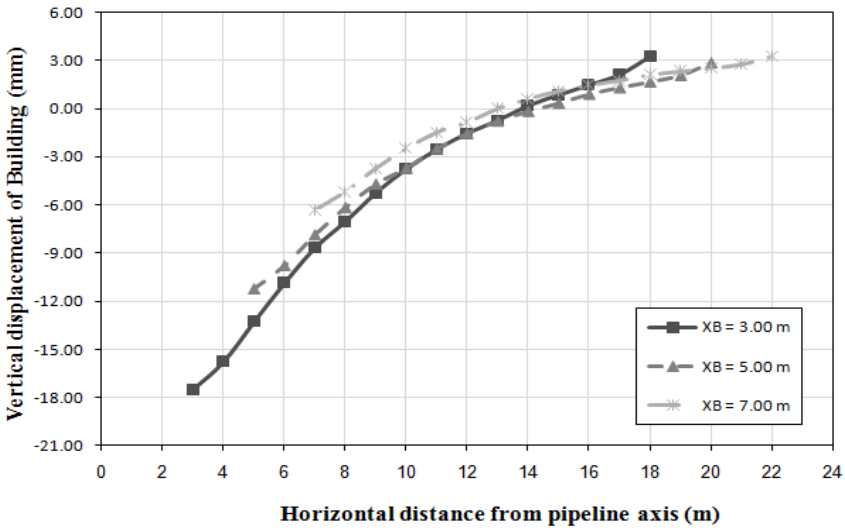

Figure 10 Effect of building location on vertical settlement of building.

Table 7 Evaluation of potential damage due to building location from pipeline settlement.

Properties	Case		
	XB=3.00m	XB=5.00m	XB=7.00m
Differential Sett.(ΔS)mm	20.72	14.04	9.59
Angle of Tilt (α) rad.	0.00138	0.00094	0.00064
Cumulative Maximum Tensile Crack Width (C_t) mm	4.45	4.79	4.47
Cumulative Maximum Principal Crack Width (C_p) mm	4.82	4.06	3.38
Damage Category	Moderate	Moderate	Moderate

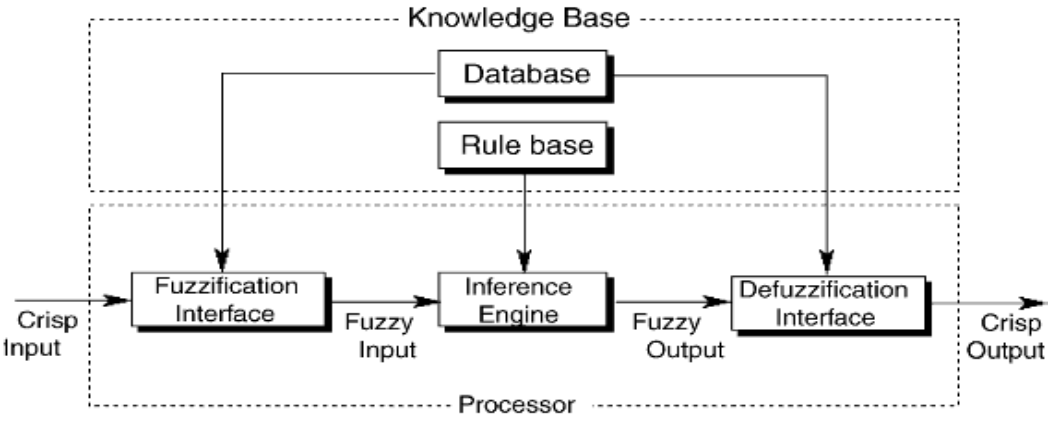

Figure 11 Fuzzy inference systems (FIS) component.

5.1 Four inputs – one output decision support tool

Fuzzy logic decision support tools (FLDST), is a control law that is described by a knowledge-based system consisting of IF . . . THEN rules with vague predicates and a fuzzy inference mechanism. The rule-base is the main part of the FLDST. It is formed by a family of logical rules that describes the relationship between the four inputs (in our case): pipeline settlement along with pipeline settlement location, building location with burial depth and the one output of the fuzzy system (damage category of building).

Based on the operator experience, the structure of the FLDST has four inputs and one output. The fuzzification and defuzzification processes are illustrated as following:

(a) Fuzzification:

Figure 12 illustrates the proposed structure of the FLDST. These inputs are the Pipeline Settlement (P.St), the Settlement Location (St.L.x), Building Location (B.L) and Burial Depth (B.D). The data obtained from ANSYS as shown at Fig. 5 describes the influence of pipeline settlement on the vertical settlement of building. The inputs of this case are the Pipeline Settlement (P.St), the Settlement Location (St.L.x) and the Building Location (B.L). The data obtained from ANSYS describes the influence of pipeline settlement, settlement location and building location on the damage of building.

The universe of discourse for the first input of FLDST is chosen from 1%D to 10%D. Five Membership Functions (MFs) are chosen for the first input (pipeline settlement) where the outer right MF is S function, the outer left is Z function, one of the inner three MFs is a trapezoidal function, and other two are represented by triangle function as shown in Fig. 13.a. The linguistic terms for defining the membership functions are: (1%D), (3%D), (5%D), (8%D) and (10%D), where %D is the percentage of settlement occurs as a function of pipeline diameter.

The data obtained from ANSYS as shown at Fig. 7 describes the influence of pipeline settlement location on the vertical settlement of building. The universe of discourse for the second input (pipeline settlement location) of FLDST is chosen from 0m to 12m. A five membership function are chosen for the second input (settlement location) where the outer right MF is S function, the outer left is Z function, and the inner three MF are represented by triangle function as shown in Fig. 13b. The linguistic variables of MFs defined as (0m), (3m), (6m), (9m), and (12m).

The data obtained from ANSYS as shown at Fig. 10 describe the influence of building location on the vertical settlement of building. The universe of discourse for the third input (building location) of FLDST is chosen from 3m to 7m. Five membership

functions are chosen for the third input (building location) where the outer right MF is S function, the outer left is Z function, and the inner three MFs are represented by gaussian function as shown in Fig. 13.c. The linguistic terms for defining the MFs are: (3m), (4m), (5m), (6m), and (7m).

The data obtained from ANSYS as shown at Fig. 8 describe the influence of burial depth on the vertical settlement of building. The universe of discourse for the fourth input (burial depth) of FLDST is chosen from 3m to 7m. Five triangle membership functions are chosen to represent linguistic variables of MF and it's defined as (3m), (4m), (5m), 6m), and (7m) as shown in Fig. 13d.

Finally, six membership functions are used to represent the linguistic variables of output (damage category of building), where the outer right MF is S function, the outer left is Z function, and the inner four MFs are represented by gaussian. The name of six linguistic variables of output are: NEG is negligible, VSL is very slight, SL is slight, MOD is moderate and SV is severe and VSV is very severe as shown in Fig. 13e.

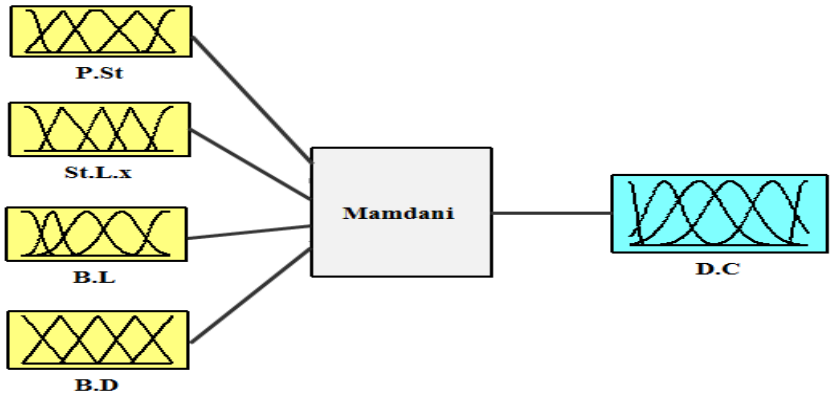

Figure 12 Structure of FLDST: 4 inputs (pipeline settlement, settlement location, building location, burial depth), 1 output (damage category).

(b) Defuzzification:

The rule base was constructed based on data obtained from ANSYS results after solving lots of cases. A sample of these rules that cover the whole range of the four parameters are introduced in Table 8.

Figure 14 illustrates one of the surface of rules in three-dimensions for the four parameters. The damage category is determined for different values of pipeline settlements as well as for different settlement locations at the fixed value of burial depth and building location. It can be shown that the value of settlement has more effect on building damage than settlement location.

Figure 15 illustrates another surface of rules in three-dimensions for the four parameters. The damage category is determined for different values of pipeline settlements as well as for different value of burial depth at the fixed value of settlement locations and building location. It can be shown that the value of settlement has more effect on building damage than burial depth.

(c)Validation of Results:

The fuzzy output as damage category was briefly validated by using numerous examples for different values for the four parameters that was chosen randomly to cover the whole range of 4 parameters, as inputs, to get the results first in fuzzy system. Then, running the same values using ANSYS and all the results were consistent in the two methods. Some of these values that were run twice were introduced in the Table 9.

These examples were run by ANSYS for different pipeline settlement along with different pipeline settlement location, different burial depths and different building location. The calculated category of damage was consistent to the results obtained from the proposed method.

Table 9 illustrates several examples from MATLAB that was validated by ANSYS computer program to validate and evaluate the proposed FLDST in evaluating the damage category of building. FLDST proved to have the ability to cover the entire range of pipeline settlement, settlement location and building location along with burial depth. Now we can use it to evaluate damage category of building at any value of entire range of inputs for accurate results without using ANSYS program and calculations.

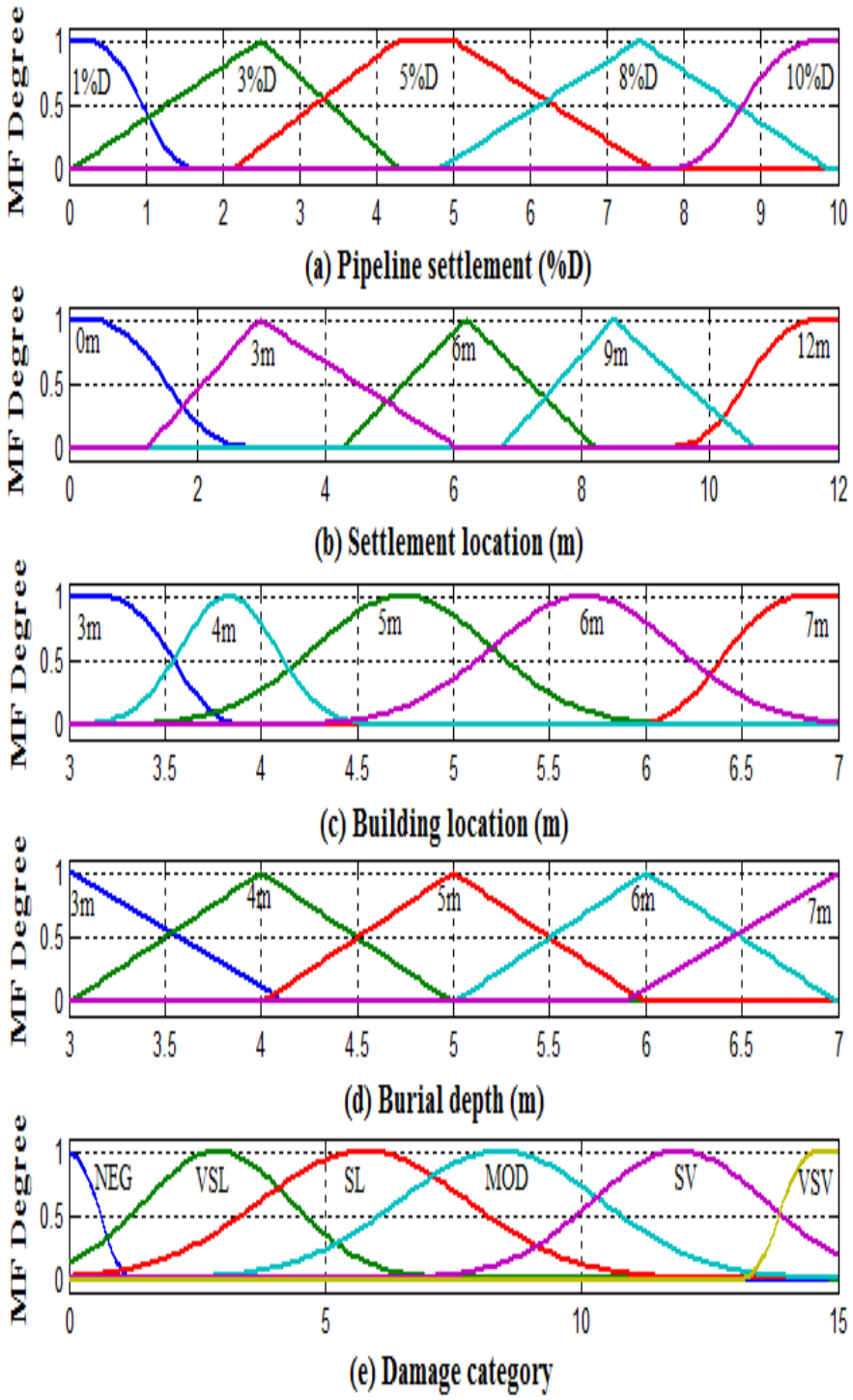

Figure 13 Membership functions inputs (a), (b), (c), (d) and output (e) of FLDST.

Table 8 Fuzzy rule base for four inputs

				Pipeline Settlement (%D)				
	XB	H_{soil}	X	1%	3%	5%	8%	10%
Building Location (XB)	3m	3m	0m	SL	MOD	MOD	SV	SV
	3m	3m	6m	SL	MOD	MOD	SV	SV
	3m	3m	12m	VSL	SL	SL	MOD	MOD
	3m	5m	0m	VSL	SL	MOD	SV	SV
	3m	5m	6m	VSL	SL	MOD	MOD	MOD
	3m	5m	12m	VSL	VSL	SL	SL	MOD
	3m	7m	0m	VSL	SL	SL	MOD	SV
	3m	7m	6m	VSL	SL	SL	MOD	MOD
	3m	7m	12m	VSL	VSL	VSL	SL	MOD
	5m	3m	0m	SL	MOD	MOD	SV	SV
	5m	3m	6m	SL	SL	MOD	MOD	SV
	5m	3m	12m	VSL	SL	SL	MOD	MOD
	5m	5m	0m	VSL	SL	MOD	SV	SV
	5m	5m	6m	VSL	VSL	SL	MOD	MOD
	5m	5m	12m	VSL	VSL	VSL	SL	MOD
	5m	7m	0m	VSL	SL	SL	MOD	SV
	5m	7m	6m	VSL	VSL	SL	MOD	MOD
	5m	7m	12m	VSL	VSL	VSL	SL	MOD
	7m	3m	0m	VSL	SL	MOD	MOD	SV
	7m	3m	6m	VSL	SL	MOD	MOD	MOD
	7m	3m	12m	VSL	VSL	SL	SL	MOD
	7m	5m	0m	VSL	SL	MOD	MOD	SV
	7m	5m	6m	VSL	VSL	SL	MOD	MOD
	7m	5m	12m	VSL	VSL	VSL	SL	MOD
	7m	7m	0m	VSL	VSL	SL	MOD	MOD
	7m	7m	6m	VSL	VSL	VSL	SL	MOD
	7m	7m	12m	VSL	VSL	VSL	SL	MOD

Burial Depth (H_{soil})

Settlement Location (X)

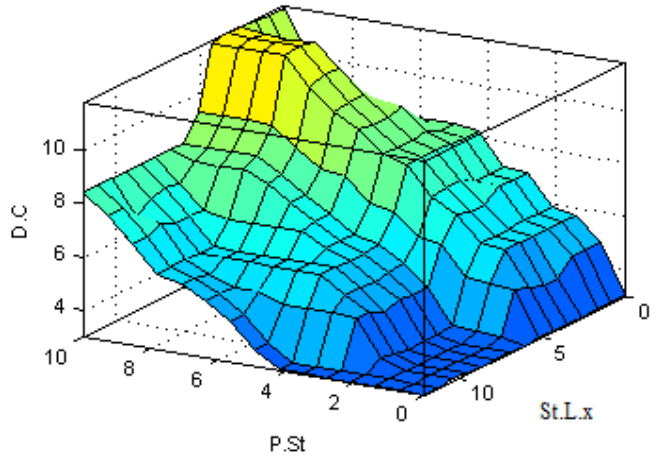

Figure 14 Damage category surface for pipeline settlement and settlement location in case of four inputs.

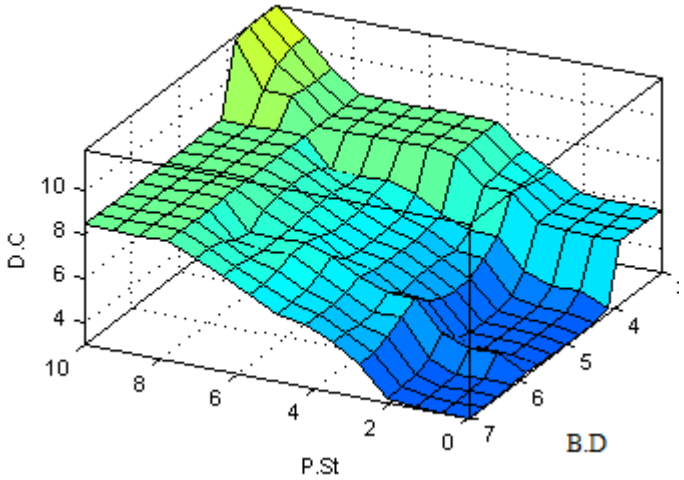

Figure 15 Damage category surface for pipeline settlement and burial depth in case of four inputs.

Table 9 Evaluation of potential damage for four parameters

	Pipeline Settlement	Settlement Location	Building Location	Burial Depth	Damage Category
IF	1.5%D	0.0m	3.0m	3.5m	SL
	3%D	3.5m	4.5m	4.5m	MOD
	4.5%D	11.5m	6.5m	6.5m	VSL
	8%D	0.0m	6.5m	4.5m	MOD
	9.5%D	7.0m	5.0m	3.0m	SV
		AND	AND	AND	THEN

6. Summary and Conclusions

The purpose of this study is to extend the research done in introducing the fuzzy logic for damage assessment of buildings due to nearby pipeline deterioration. The main contribution in this research is applying up to four different parameters of pipeline deterioration at the same time. This requires the use of Matlab to build synchronized four membership functions as input functions and a huge number of rule bases which play the role of experts in the decision. By using data from the major sewer pipeline projects in Egypt and detecting the main causes of failure. We choose here four parameters (pipeline settlement, settlement location, building location and burial depth). It can be concluded from this research that:

1. Fuzzy decision support tool is a very efficient and powerful tool for evaluating the damage

categories of buildings due to different parameters of sewer pipeline failure. This is time saving and provide a guide for less experienced engineers.
2. Using fuzzy logic for studying the effect of four different parameters of pipeline deterioration at the same time on the damage of nearby buildings helps to evaluate the weight of each parameter with respect to the others.
3. By including the four mentioned parameters of pipeline failure. It was found that the value of pipeline settlement has the major impact on the damage of adjacent buildings, more than the settlement location, the building location and the burial depth.
4. Also, we can add that, following the pipeline settlement, the settlement location has more effect on building damage followed by the burial depth, then the building location.

Potential studies:

a. The use the Genetic Algorithm (GA) to optimize the parameter of FDST will be very useful in minimizing the error according to defined fitness function. Also, the use of the neural network for design system like FDST in civil engineering will be good extension to this research.
b. Build new fuzzy decision support tool by using new data in the practical range for pipes, soil properties and dimensions to provide a database library to predict potential damage in surrounding buildings in existing and future sewer pipeline projects.
c. Fuzzy expert system is flexible to enter other parameters with different ranges for other applications in civil engineering.

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References

[1]Swanson, P.G. (2007). ANSYS inc. theory, Theoretical Manual, Release 11, U.S.A.
[2]Aye, Z.Z. (2006). Ground movement prediction and building damage risk-assessment for the deep excavations and tunneling works in bangkok subsoil, International Symposium on Underground Excavation and Tunneling, Bankok, Thailand.
[3]Peck, R.B. (1969). Deep excavation and tunneling in soft ground, 7th ICSME, State of the Art, (3):225–290, Mexico City.
[4]Clough, G.W. (1990). Construction induced movements of in-situ walls, ASCE Geotechnical Special Publication, (25):439–470.
[5]Burland, J.B. (1977). Behavior of foundations and structures, SOA Report Session 2, 9th International Conference, SMFE, 495–546, Tokyo.
[6]Boscarding, M.D., and Cording, E.G. (1989). Building response to excavation-induced settlement, Journal of Geotechnical Engineering, ASCE, 115(1):1–21.
[7]Metwally, K.G. (2009). Damage assessment of buildings due to deterioration of pipelines using FEM and GIS, Ph.D, Thesis, Dept. of Structural Eng., Cairo University, Cairo, Egypt.
[8]Zadeh, L.A. (1965). Fuzzy sets, Information Control, 8(3):38–53.
[9]Burak, G.A. (2008). Fuzzy decision support system to determine swell/shrink factor affecting earthwork optimization of highways, Mathematical and Computational Applications, 13(1):61–70.
[10]Ross, T.J. (1995). Fuzzy logic with engineering applications, McGraw Hill Co, New York.
[11]Cox, E. (1999). The fuzzy systems handbook, Second Edition, Academic Press, California,.
[12]Pourzeynali, S., Lavasani, H.H., and Modarayi, A.H. (2007). Active control of high rise building structures using fuzzy logic and genetic algorithms, Engineering Structures, 29:346–357.
[13]Aldawod, M., Samali, B., and Naghady, F. (2001). Active control of along wind response of tall building using a fuzzy controller, Engineering Structures, 23(15):12–22,
[14]Ha, Q.P. (2001). Active structural control using dynamic output feedback sliding, Australian conference on robotics and automation, Sydney, 14–25.
[15]Symans, M.D., Kelly, W. (1999). Fuzzy logic control of bridge structures using intelligent semi-active seismic isolation systems, Earthq. Eng. Struct. Dyn., 28:37–60.
[16]Lin, Y., Cheng, C., Lee, C. (2000). A tuned mass damper for suppressing the coupled flexural and torsional buffeting response of long-span bridges, Engineering Structures, 22(1):195–204.
[17]Ahlawat, A.S., Ramaswamy, A. (2001). Multi objective optimal structural vibration control using fuzzy logic control system, Engineering Structures, 127(11):1330–1337.
[18]Emarah, D.A., Hussein, M.M., Mousa, H.M. and Akl, A.Y. (2011). Evaluation of buildings damage due to different parameters of pipeline failure using fuzzy decision support tool, Journal of American Science, 7(4): 374-384.
[19]Mamdani, E.H., and Assilian, S. (1975). An experiment in linguistic synthesis with fuzzy logic controller, International Journal of Man-Machine Studies, 7:1–13.
[20]Takagi, T., and Sugeno, M. (1985). Fuzzy identification of systems and its applications to a fuzzy logic controller, IEEE Transactions on Systems Man Cybernetics, 15:116–132.

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Comparison of Mental Health in Male Adolescents with and without Internet Addiction

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Abstract: In presented study, mental health of male adolescents with and without Internet Addiction is compared.. Participants included 114 male adolescents, selected by purposive sampling method from Internet cafe` of Bandar Abbas which 55 individuals in group with Internet Addiction and 59 individuals in group without Internet Addiction were assigned. Data collecting tools of the research were including Young's Internet Addiction Test (IAT), and General Health Questionnaire (*GHQ-28*). The Independent Samples T Test was used to analyze test research hypothesis. Results indicated that adolescents with Internet Addiction in the overall mental health and Somatic symptoms, Anxiety/insomnia, Social dysfunction and severe depression experience had more problems significantly**.** Findings and implications for health adolescents are discussed. [Abbas Ebrahimi, Zoleikha Sadeghi. **Comparison of Mental Health in Male Adolescents with and without Internet Addiction.** Life Science Journal. 2011;8(4):609-612] (ISSN:1097-8135). http://www.lifesciencesite.com.

Keywords: Internet Addiction Disorder (IAD), Mental Health, Adolescents.

1. Introduction

Sine 1976 which internet has born in has become one of the most advanced technologies of today's world. People's simple access to this technology and being anonymous in this vast world are the distinctive characteristics that transformed this tech to one of the most commonly used technologies of the world.

Internet can be used in field of learning, educating, research project, human relation and personal interest. However, these characteristics which are the advantages of this technology can become the reason of trauma and mental problems in case of excessive usage (Greenfield, 2004). Internet pathological use causes some behavioral problems which are alike the symptoms of other addiction disorders (OReilly et al., 1996). In recent years with increasing the access to internet in Asian countries and of course in Iran , dependency and addiction to excessive internet use has increased and cause trauma and lots of mental disorder in users (Chou and Hsiao, 2000). Internet addiction is a modern addiction which is the outcome of easy access to the computers and online information. Peoples who suffer this kind of addiction can have signs and symptoms of those who are addicted to alcohol, gambling, drugs, or other Obsessive – compulsive behaviors. A way to describe the people who suffer this kind of disorder is, for such people virtual environments are more attractive than the real world. This addiction has affected millions of Americans and their families in different aspect of their lives such as school, family, work and social relations (Young, 1998).

Conducted studies have reported the prevalence of 0.3% to 22% (Vizeshfar, 2005; Kim et al., 2006). The relation of internet addiction and mental health disorders has studied in different research (Yen et al., 2007; Mythily et al., 2008). According to one of these studies, internet addictions are usually alone, isolated and shy and suffer symptoms of depression (Niemz et al., 2005).

Other studies have shown that there is a relation between internet addiction and symptoms of depression, low self-confidence, anxiety, sociophobia, and having problem in education and work of the users. Park and Song have also linked internet addiction with number of friends, momentums, sensitivity in their relation with others and self efficiency (Park and Song, 2002). Lee et al. (2001) reported that anxiety, depression, self-effacement in internet addicts. Whang et al. (2003) have also implied compulsive behavior, depressed mood and sense of loneliness in internet addicts. Therefore according to available evidence from researches, the purpose of present study is surveying the mental problems and traumas among internet addicts in comparison with non-addicts. For this purpose a comparison has been made in a research between two groups about the Somatic symptoms, anxiety/insomnia, social dysfunction, and severe depression. On this basis, research hypothesis are:

- There was a significant difference in general health between internet addicts and non addicts.
- There was a significant difference in Somatic symptoms between internet addicts and non addicts.
- There was a significant difference in level of anxiety/insomnia between internet addicts and non addicts.

- There was a significant difference about Social dysfunction between internet addicts and non addicts.
- There was a significant difference of severe depression between internet addicts and non addicts.

2. Material and Methods

This is a comparative study which all of the information has collected with survey method. The study sample is a group of 114 male adolescent from Bandar-Abbas (southern city of Iran which home Bandar Abbas port) which has chosen with purposive sampling method. The ranges of samples age were between 17 to 24 years old with the Mean age of 19 and standard deviation of 2.6. we went to internet café for selecting samples and after describing the purpose of research for the participants and explaining their right to probable withdrawal and the privacy policy of the research, the questionnaire of internet addiction was distributed between the youngster users of the internet and after obtaining scores of the samples, they were classified intro internet addicts and non-addicts. Accordingly 55 individuals (48.2%) were addicted and 59 (51.8%) were not addicted to internet. for gathering the data about internet addiction, Young's Internet Addiction Test, was used (Young, 2004). This questionnaire had 20 items and its answering scale was 5 degree Likert which scored from 1 (rarely) to 5 (always) .The score range was between 20 to 100 and higher scorers indicate more dependency to the internet. In order to divide the samples into 2 groups of addicts and non-addicts, the cut-off point of 49 was choosed. Yoo et al. (2004) used Cronbach's alpha method and have reported the reliability coefficient of this test equal to 0.90. In present study the reliability coefficient of the test was 0.86 according to Cronbach's alpha method. For evaluation of mental health, the Scaled General Health Questionnaire-28 (GHQ-*28*) was used (Goldberg and Hillier, 1979). The questions of this questionnaire were analyzing the mental condition of participant in the last 1 month and include symptoms of abnormal thoughts and feeling and aspects of observable behavior and stresses on the situation of here and now. This questionnaire consists of four sub-scales and each sub-scale consists of 7 questions. Questions 1 to 7 were related to physical symptoms sub-scale, 8 to 14 were related to anxiety/insomnia sub-scale, 15 to 21 were related to social dysfunction sub-scale and 22 to 28 were related to severe depression sub-scale. The method of answering the questions was according to 4 degree Likert which varies from 0 to 3.There are two kind of scoring for this questionnaire. In The first method, questions are graded as 0,0,1,1. In this method scores varies between 0 to 28 .Second method is the Likert Grading Method which a score of 0 to 3 is dedicated to each question and the score of test is varied between 0 to 84. In both of the grading methods, lower score indicate higher mental health and in this study second method has been used. Taghavi has mentioned the reliability of the test with 3 method of Test-Retest, Split-half and Cronbach's alpha method in Iran, 0.70, 0.93 and 0.90 respectively. The validity of the general health questionnaire in Iran also reported at the appropriate level by using 3 methods of Concurrent validity, sub test correlation with total score and Factor Analysis. (Taghavi, 2000) In present study in order to evaluating the reliability of test, Cronbach's alpha and Test-Retest have been used which reliability coefficient of this questionnaire with Cronbach's alpha coefficient is equal to 0.88 and by using the Test-Retest method it is equal to 0.89 . In the section of descriptive Statistics, indexes of mean and standard deviation were calculated and the Independent Samples T Test was used for testing the hypothesis of the study .The calculations was made by SPSS ver.16 statistic software.

3. Results

At first a statistical analysis was done to check the study's hypothesis. to do so , mean and standard deviation of each of variables was calculated which is presented in Table 1.

Table 1. Mean and standard deviation of the variables of the study

Variable	Group	N	Mean	Standard Deviation
Scores of IA questionnaire	With IA	55	78	4.78
	Without IA	59	28	3.21
Score of general health	With IA	55	60.38	6.94
	Without IA	59	26.05	5.84
Somatic symptoms	With IA	55	14.81	2.34
	Without IA	59	6.18	1.98
Anxiety/insomnia	With IA	55	15.49	2.48
	Without IA	59	5.86	1.46
Social dysfunction	With IA	55	14.89	2.43
	Without IA	59	7.35	2.46
Severe depression	With IA	55	15.18	2.22
	Without IA	59	6.64	2.51

IA= Internet addiction

As earlier mentioned Independent Samples T Test was used to test the hypothesis. First hypothesis of study was implied to a significant difference of scores in general health between two groups of internet addicts and non-addicts which for evaluating the hypothesis, T test value was equal to 28.62 with 112 degree of freedom in level of 0.001. Detailed findings have presented in Table 2

Table 2. Mean score comparison of two groups of internet addicts and non-addicts in general health

Group	N	Mean	Mean Difference	T value	DF	P
With IA	55	60.38	34.33	28.62	112	0.001
Without IA	59	26.05				

IA= Internet addiction

Second hypothesis was implying to the significant difference of Mean scores of Somatic symptoms between two groups of internet addicts and non addicts which for evaluating the hypothesis, T test value was equal to 21.22 with 112 degree of freedom in level of 0.001. Detailed findings have presented in Table 3

Table 3. Mean score comparison of two groups of internet addicts and non-addicts in Somatic symptoms

Group	N	Mean	Mean Difference	T value	DF	P
With IA	55	14.81	8.63	21.22	112	0.001
Without IA	59	6.18				

IA= Internet addiction

Third hypothesis was implying to the significant difference of Mean scores of anxiety/insomnia between two groups of internet addicts and non addicts which for evaluating the hypothesis, T test value was equal to 25.38 with 112 degree of freedom in level of 0.001. Detailed findings have presented in Table 4.

Table 4. Mean score comparison of two groups of internet addicts and non-addicts in anxiety/insomnia

Group	N	Mean	Mean Difference	T value	DF	P
With IA	55	15.49	9.62	25.38	112	0.001
Without IA	59	5.86				

IA= Internet addiction

Fourth hypothesis was implying to the significant difference of Mean scores of social dysfunction between two groups of internet addicts and non addicts which for evaluating the hypothesis, T test value was equal to 16.40 with 112 degree of freedom in level of 0.001. Detailed findings have presented in Table 5.

Table 5. Mean score comparison of two groups of internet addicts and non-addicts in social dysfunction.

Group	N	Mean	Mean Difference	T value	DF	P
With IA	55	14.89	7.53	16.40	112	0.001
Without IA	59	7.35				

IA= Internet addiction

Fifth hypothesis was implying to the significant difference of Mean scores of depression between two groups of internet addicts and non addicts which for evaluating the hypothesis, T test value was equal to 19.15 with 112 degree of freedom in level of 0.001. Detailed findings have presented in Table 6.

Table 6. Mean score comparison of two groups of internet addicts and non-addicts in depression

Group	N	Mean	Mean Difference	T value	DF	P
With IA	55	15.18	8.53	19.15	112	0.001
Without IA	59	6.64				

IA= Internet addiction

4. Discussion

Present study has been made with the purpose of analyzing and comparing the general health of those who are addicted to the internet and those who are not, and the final result was verified the hypothesis of the study. Accordingly in general health and also in indexes of Somatic symptoms, anxiety/insomnia, social dysfunction and depression ,those who were addicted to the internet get higher scores significantly, in other word internet addicts have more health problem in comparison with other group i.e. non-addicts. In four components of general health addicted group, suffer more health problems and The findings was consistent with the previous studies **(**Yen et al., 2007; Mythily et al., 2008; Lee et al., 2001; Morgan and Cotton, 2003).

Findings of the present study had useful practical implications for prevention and treatment of internet addiction for physicians, psychiatrists and counselors. Internet addiction is a modern addiction which we are getting to know it's dimensions more and more every day. Relationship of this variable with other variable like personality characteristics, identity styles and other kind of addictions could be appropriate subjects of research for future studies. In addition, before generalizations it has to be considered that sample group was limited to male sex and questionnaire was self-reporting.

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References

1- Greenfield D. Virtual Addiction. Siahate Gharb J 2004; 18:18-26.
2- OReilly M. Internet addiction: a new disorder enters the medical lexicon. CMAJ. 1996; 154(12):1882-1883.
3- Chou C, Hsiao MC. Internet addiction, usage, gratification, and pleasure experience: the Taiwan college students' case. Comput Educ. 2000; 35(1):65-80.
4- Young KS. Caught in the net: how to recognize the signs of Internet addiction and a winning strategy for recovery. New York: John Wiley & Sons; 1998.
5- Kim K, Ryu E, Chon MY, Yeun EJ, Choi SY, Seo JS, et al. Internet Addiction in Korean Adolescents and its Relation to Depression and Suicidal Ideation: A questionnaire survey. Int J Nurs Stud, 2006; 43(2):185-92.
6- Vizeshfar F. Assessment of the internet addiction between Larian net users. Journal of fundamentals of mental health, 2005;7(25-26):27-33.
7- Yen JY, Ko CH, Yen CF, Wu HY, Yang MJ. The comorbid psychiatric symptoms of Internet addiction: attention deficit and hyperactivity disorder (ADHD), depression, social phobia, and hostility. J Adolesc Health, 2007;41(1):93-8.
8- Mythily S, Qiu S, Winslow M. Prevalence and correlates of excessive Internet use among youth in Singapore. Ann Acad Med Singapore 2008; 37(1):9-14.
9- Niemz K, Griffiths M, Banyard P. Prevalence of pathological Internet use among university students and correlations with self-esteem, the General Health Questionnaire (GHQ), and disinhibition. Cyberpsychol Behav, 2005; 8(6):562-70.
10- Park YS, Song HJ. The psychological characteristics of juveniles regarding internet addiction. WebHealth Research, 2002; 5: 1–15.
11- Lee SB, Lee KK, Pail KC, Kim HW, Shin SK. Relationship between internet addiction and anxiety, depression, and self efficacy in middle and high school students. Journal of the Korean Neuropsychiatry Association, 2001; 40(6): 1174–1184.
12- Whang LS, Lee S, Chang G. Internet over-users' psychological profiles: a behavior sampling analysis on Internet addiction. CyberPsychology and Behavior, 2003; 6 (2): 143–150.
13- Young KS. Center for online Addiction-Health and Resources for Internet Addicts. 2004; November 24.
14- Yoo HJ, Cho SC, Ha J, et al. Attention deficit hyperactivity symptoms and Internet addiction. PCN. 2004; 58: 487- 494.
15- Goldberg DP, Hillier VF. A scaled version of the General Health Questionnaire, Psychological Medicine, 1979; 9: 139-145.
16- Taghavi MR. Validity and Reliability of the General Health Questionnaire, Psychology Journa, 2000; 20: 381-398.
17- Morgan C, Cotten S. The relationship between Internet activities and depressive symptoms in a sample of college freshmen. CyberPsychology and Behavior 2003; 6(2): 133–142. NTIA and the Economics and Statistics Administration, 2002.

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