CONTENTS

Faculty leadership through self-assessment.

Dr. S.Srividhya [1], P. Viji [2]

[1.] Assistant Professor, School of Management Studies, Anna University,Regional Center Coimbatore, Jothipuram Post, Coimbatore – 641 047, Tamilnadu, India. Email: ssautcbe@gmail.com

[2.] Ph.D Research Scholar, School of Management Studies, Anna University,Regional Center Coimbatore, Jothipuram Post, Coimbatore – 641 047, Tamilnadu, India. Email: srpviji@yahoo.com

Abstract : Basically, an assessment on faculty's performance involves assessing of competencies in needed domains of the profession. Indeed, outside of the teaching competencies and different skilled responsibilities that facilitate outline what makes a good faculty; a college should even have sure traits or characteristics that area unit imperative to form his or her teaching effective. The absence or lack of such traits might spell the distinction between success and failure in transfer regarding the specified learning outcomes in students. The performance of the faculty is also influenced by several factors like family and monetary background; expertise and exposure. For this study, 168 faculty members were hand-picked from the engineering schools attached to Anna University of Technology, Coimbatore, across nine zones. A group of visible and invisible competencies were derived from previous studies. "T" check and confirmative co relational analysis were made to make sure the validity and dependability of the constructs. A positive association with age, family size and family financial gain and also the competencies possessed were found.

[Dr. S.Srividhya, P. Viji. **Faculty leadership through self-assessment.** *Life Sci J* 2012;9(4):4774-4778] (ISSN:1097-8135). http://www.lifesciencesite.com. 717

Key words: *Faculty performance, self-assessment, visible competencies, invisible competencies.*

Introduction

A teaching process can be viewed as a well thought of series of steps or action to facilitate learning and teaching effectiveness can be measured by the degree to which the expected learning takes place (De La Rosa, 2001). Danielson and Mc Greal (2000) proposed a model containing four domains embodying the components of specialized practice. These are planning and training, the classroom location, instruction, and specialized responsibilities. This model highlights the fact that faculty's functions and responsibilities are varied and encompasses several areas of competencies. Competencies in these domains can serve as criteria of faculty's performance and effectiveness. Meanwhile, Tigelaar, et al., (2004) proposed an outline of teaching effectiveness with the following major domains – person as a faculty, expert on content knowledge, facilitator of knowledge processes, organizer, and scholar/all-time student. The most important addition in this outline is the authors' giving importance on aspects of a faculty's personality that are conducive to learning by proposing the domain of 'person as a faculty'.

Self assessment in teacher performance

An alternative approach in assessing teacher performance is self-assessment – where faculty's rate and evaluate themselves based on a well-defined set of competencies or characteristics. Nhundu (1999) argues that self-evaluation have the greatest potential of producing changes in teaching practice because they provide teachers with the rare opportunity to reflect on their teaching and modify accordingly. Ross and Bruce (2005) projected a model of self-assessment comprised of three processes:

i. Self-observation
ii. Self-judgment,
iii. Self-reaction.

Objectives of the study

1. To reveal the profile of the teachers chosen for the study.
2. Exhibit the self-assessment of faculty's competencies among them.
3. Association between the profile of the faculties and visible and invisible competencies.

Need for the Study

While studying the competency mapping of faculties, it is imperative to select the faculty working in various engineering colleges since there is a mushroom growth of engineering colleges and also the number of faculty working. A portion of young post-graduates in engineering get their employment at various engineering colleges. They study only technical papers in their curriculum, but not pedagogy. Since the faculty members are fresh and lesser experienced, they are struggling to enrich their competencies according to the need of their stakeholders. Hence, this critical area has been chosen for research to provide a solution to enrich their competencies.

Research methodology

A sample size of 168 teachers were selected using simple random sampling technique, from the population of 168 engineering colleges affiliated to Anna University of Technology, Coimbatore. An Interview Schedule was used for the study.

Tools for analysis

1. Confirmatory factor analysis was administered to examine the reliability and validity of the variables included in each construct.
2. 'T' test has been administered to find out the significant difference between the two means among the male and female stakeholders in the colleges.
3. One way analysis of variance has been applied to analyze the association between the profile of the faculty and their views on the various competency dimensions.

In this paper, two important aspects have been discussed. One is invisible competencies which have equipped skills, administration, headship, people skills, principled and social responsibilities, participative and scholarship ethnicity, directorial skills, industry knowledge and self-effectiveness. Another is visible competencies which include journal publication, professional presentation, instructional method, institutional support, professional service and college/university service.

Initially, the background of the faculties has been examined with the help of their important profiles. The important profiles included are gender, age, marital status, and nature of family, family size, number of dependent population, years of experience, personal earnings per month, and family earnings per month.

Invisible Competencies among the faculties

The highly possessed variable in management leadership among the male and female faculties is helps in making action plans and support to achieve organization objectives since their mean scores are 3.9884 and 3.7719 in that order. On the topic of the level of variables in management leadership, the significant difference among the male and female faculties has been identified in the case of taking more research oriented activities. Since its T-statistics is significant at five percent stage. The extremely view variables in peoples skills among the male and female faculties is encouraging high performance since their mean scores are 3.7332 and 3.2083 respectively.

In the case of ethical and social responsibilities, these are inculcates social orientation and inspire to take initiatives since their mean scores are 3.8884 and 3.1539 respectively. Regarding the possession of participative and learning culture, the highly possessed variables among the male and female faculties are to facilitate open communication and facilitating improvements in performance since their mean scores are 3.7085 and 3.2664 correspondingly. Regarding the level of possession of variables in people skill, ethical and social responsibilities and participative and learning culture, the significant difference among the male and female faculties have been noticed in all variables included in the above said three dimensions. The results are shown in the table-1.

Table-1: Self assessment on invisible competencies among the faculties (SAICF)

S.No	Variables in SAICF	Mean score among		T-statistics
		Male	Female	
I	**Management leadership**			
1	Helps in making action plans	3.9884	3.6082	1.5441
2	Promotes goal setting process	3.7032	3.4501	1.6088
3	Support to achieve organizational objectives	3.6639	3.7719	-0.3889
4	Encourage frank discussions	3.7144	3.4332	1.4991
5	Take more research oriented activities	3.6603	3.2115	2.0165*
II	**People skills**			
1	Periodic performance appraisal	3.4432	3.0884	2.1773*
2	Strengthening relationship with peer groups	3.6609	3.1176	2.3508*
3	Encouraging high performance	3.7332	3.2083	2.6676*
III	**Ethical and social responsibilities**			
1	Inculcates social orientation	3.8884	3.0446	3.4517*
2	Inspires to take initiatives	3.7032	3.1559	3.5083*
3	Honesty in implementation of program	3.6568	3.1108	3.0117*
IV	**Participative and learning culture**			
1	Participative approach in designing T&D plans	3.5508	3.0084	2.8586*
2	Facilitating improvements in performance	3.6673	3.2664	2.1447*
3	Facilitate open communication	3.7085	3.1089	2.7667*

*Significant at five percent level.

The highly possessed variable in organizational skills among the male and female faculties is promotes the growth and learning and promotes transparency since their mean scores are 3.7309 and 3.5887 respectively. Regarding the industrial knowledge these variables are provision of consultation activities and participation and arrangement of MOU with industries since their mean scores are 3.8646 and 3.2146 respectively. In the case of self effectiveness, these variables are assessing the self development plans since their mean scores are 3.4177 and 2.7336 respectively. Regarding the possession of variables the significant difference among the male and female faculties has been noticed in the possession of all variables included in industry knowledge, prepared skills and self efficiency. The results are shown in table 2.

Table-2: Self-assessment on invisible competencies among the faculties (SAICF)

S.No	Variables in SAICF	Mean score among		T-statistics
		Male	Female	
V	**Organizational skills**			
1	Promotes transparency	3.3446	3.5887	-0.7389
2	Helps to set objectives	3.6607	3.3816	1.3865
3	Promotes the growth and learning culture	3.7309	3.2117	2.4508*
VI	**Industrial knowledge**			
1	Arrangement of industry tie up programs	3.6608	3.0218	2.5991*
2	Participation in institutional building activities	3.7334	3.1771	2.7667*
3	Provision of consultation activities	3.8646	3.2084	2.8081*
4	Participation and arrangement of MOU with industries	3.7029	3.2146	2.5084*
VII	**Operational skills**			
1	Knowing the SWOT of institutions	3.8441	3.0864	3.1171*
2	Knowing the way to improve faculties competencies	3.4667	3.1133	1.6646
3	Promotion of creativity and innovations in Pedagogy	3.5087	2.9087	2.7817*
IV	**Self –effectiveness**			
1	Assessing the self-development needs	3.3088	2.6605	2.9108*
2	Assessing the self-development plans	3.4177	2.7336	3.0911*
3	Implementation of self-appraisal	3.2667	2.5085	3.1787*
4	Implementation of self-correction practices	3.3085	2.6887	3.0942*

*Significant at five percent level.

Association between the profile of faculties and their possession of invisible competencies

Regarding the possession of management leadership, the significantly associating profile variables are marital status, number of dependent population, years of experience and family income whereas in the possession of people skills, these profile variables are age, number of dependent population, years of experience and personal income per month. The significantly associating profile variables with the possession of ethical and social responsibility are age, years of experience, personal income per month and family income per month, whereas regarding the possession of participative and learning culture, the profile variables are personal income and family income per month. The results are given in table-3.

Table 3: Association between profile of faculties and their invisible competencies

S.No.	Profile variables	F Statistics			
		Management leadership	People skills	Ethical and Social responsibility	Participative and learning culture
1	Age	2.5889	2.7887*	2.8085*	2.3664
2	Marital status	3.1178*	2.4518	2.0339	2.7317
3	Nature of family	3.0093	3.3887	3.5884	3.2088
4	Family size	2.1173	2.5082	2.4482	2.1997
5	Number of dependent population	3.2344*	3.6677*	2.9969	2.8734
6	Years of experience	2.6679*	2.8242*	2.7069*	2.1183
7	Personal income per month	2.0442	2.6096*	2.6993*	2.5083*
8	Family income per month	2.6649*	2.2144	2.8868*	3.0989*

*Significant at five percent level

Regarding the possession of organizational skills, significantly associating profile variables is personal income per month whereas regarding the possession of industry knowledge, the significantly associating profile variables are family size and years of experience. The significantly associating profile variables with the possession of operational skills are number of dependent population, years of experience and family income per month whereas

regarding the possession of self-effectiveness, these profile variables are age, number of dependent population, years of experience and family income per month. The results are given in table-4.

Table 4: Association between profile of faculties and their invisible competencies

S.No	Profile Variables	Organizational skills	Industry knowledge	Operational skills	Selfw-effectiveness
1	Age	2.0097	2.3446	2.5417	2.7394*
2	Marital status	2.1173	2.6544	2.8603	2.9143
3	Nature of family	3.0849	3.3817	3.5209	3.6674
4	Family Size	2.4146	2.7997*	2.1177	2.3367
5	Number of dependent population	2.6334	2.9336	3.0991*	3.3996*
6	Years of experience	2.0244	2.8968*	2.9945*	3.1147*
7	Personal income per month	2.4667*	2.1089	2.2682	2.2996
8	Family income per month	2.1143	2.0667	2.4587*	2.5889*

*Significant at five percent level.

Visible Competencies among the faculties:

The visible competencies among the faculties are the competencies which can be explicitly seen by others or the competencies which can be proved by with the certificates. The visible competencies among the faculties have been measured under six dimensions. The results are given in table 5.

The highly possessed variable in journal publication among the male and female faculties is books reviewed and non-referred publication since their mean scores are 3.1788 and 3.1789 respectively. Regarding the possession of the variables in journal publication, the significant differences among the male and female faculties are seen in the case of book publication and book reviewed since their 'T' statistics are significant at five percent level. In the case of professional presentation, the significant difference among the male and female faculties has been identified in the case of national, regional and state/local conferences.

The highly possessed variable in instructional method among the male and female faculties is student's evaluation since their mean scores are 3.6441 and 3.1942 respectively. The significant difference among the male and female faculties has been noticed in the possession of two variables in it. Regarding the possession of instructional support, the highly possessed variable among the male and female faculties are students organization participated and Ph.D., committees since their mean scores are 3.5441 and 3.0092 respectively. The significant difference among the male and female faculties has been found in the possession of all three variables in it.

The highly possessed variable in the professional service among the male and female faculties is meeting activities since their mean scores are 3.4543 and 2.9969 respectively. The significant difference among the male and female faculties has been noticed in meeting activities, elected position and honors received. The highly possessed variable in college /university service among male and female faculties is college committees since their mean scores are 3.8188 and 3.5886 respectively. Regarding the possession of variables in college/university service, a significant difference among the male and female faculties has been seen in university committees.

Table 5: Self-assessment of variables on visible competencies among faculties (SAVCF)

S.No	SAVCF	Mean score among faculties in		T-statistics
		Male	Female	
I	**Journal Publication**			
1	Referred publication	2.2667	2.0688	0.6814
2	Non-Referred Publication	3.0996	3.1789	-0.7797
3	Book publication	3.1448	2.6841	2.0446*
3	Chapters publication	3.0247	2.7347	1.3456
4	Book reviewed	3.1788	2.6024	2.1779*
II	**Professional Presentation**			
1	International Conferences	2.9884	2.6436	1.2147
2	National Conferences	3.1667	2.4887	2.4334*
3	Regional Conferences	3.3089	2.9024	1.9969*
4	State/Local conferences	3.5508	3.0149	2.1144*
III	**Instructional Method**			
1	Students Evaluation	3.6441	3.1942	2.6842*
2	New Courses taught	3.3997	2.8609	2.3919*
IV	**Instructional Support**			
1	Students Organization participated	3.5441	2.9081	2.7319*
2	Ph. D. Committees	3.4992	3.0092	2.4089*
3	M.S. Committees	3.3809	2.7459	2.0667*

V	**Professional Service**			
1	Service related activities	2.6671	2.4508	0.6447
2	Meeting activities	3.4543	2.9969	2.3891*
3	Elected positions	3.1708	2.5641	2.5667*
4	Honors received	3.0996	2.5049	2.4118*
VI	**College/University Service**			
1	College Committees	3.8188	3.5886	0.9667
2	University Committees	3.0242	2.4544	2.3818*

*Significant at five percent level.

Association between the profile of faculties and their level of visible competencies:

Regarding the possession of journal publication related competencies, the significantly associating profile variables are age, number of dependent population, years of experience and family income whereas in the possession of professional presentation, these variables are age and years of experience. The significantly associating profile variables in the possession of instructional method are age, years of experience and family income whereas in the possession of instructional support, it is family income. Regarding the possession of professional service, the significantly associating profile variables are age, number of dependent population, years of experience and family income whereas in the possession of College/University service, these are age and family size. The results are given in table 6.

Table 6: Association between profile of faculties and their visible competencies (SAVCF)

S.No	Profile variables	Journal publication	Professional presentation	Instructional method	Instructional support	Professional service	College/Univsersity service
1	Age	2.88*	2.99*	2.66*	1.85*	2.90*	2.91*
2	Marital status	2.01	1.86	2.4	2.51	2.67	2.34
3	Nature of family	3.45	3.04	3.27	3.39	3.55	3.48
4	Family size	2.48	2.00	2.51	2.11	2.31	2.96*
5	Number of dependent population	3.14*	2.51	2.22	2.66	3.79*	2.06
6	Years of experience	2.45*	2.68*	2.59*	2.05	2.80*	2.91*
7	Personal income per month	2.03	2.21	2.08	1.99	2.11	1.84
8	Family income per month	2.44*	2.05	2.66*	2.58*	2.84*	2.07

*Significant at five percent level.

Conclusion and suggestions:

Both invisible and visual competencies of the colleges square measure equally vital. Whereas for measuring the invisible competencies, fair hearing is crucial. The judgment supported proof ought to be equally treated to avoid personal prejudices, biased results. The linkage between competency of the faculties and their career accomplishment may also be evaluated. Establishment of Key Performance Indicators may be created.

Corresponding Author:
Dr. S.Srividhya
Assistant Professor, School of Management Studies,
Anna University,Regional Center Coimbatore,
Jothipuram Post, Coimbatore – 641 047, Tamilnadu,
India. Email: ssautcbe@gmail.com

References:

1. De La Rosa P (2001),"Research as an Essential Element of Effective Instruction", Ad very tatenm, 1(1), pp: 54-80.
2. Danielson C and Mc Greal, T.C (2000), "Faculty's Evaluation to Enhance Professional Practice (Electronic Version), Princeton, New Jersey: Educational Testing Service.
3. Tigelaar D, Dolmans D, Wolfhagen, and Van Der Vleuten C, (2004), "The Development and Validation of a Framework for Teaching Competencies in Higher Education", Higher Education, 48(2), pp: 253-268.
4. Nhundu, T.J (1999), "Assessing Faculty Performance: A Comparison of Self And Supervisor Rating On Leniency, Halo, and Restriction of Range Errors", Zamperia, 26(1), pp: 35 – 53.
5. Ross, J and Bruce, C (2005), "Teaching Self-Assessment: A Mechanism For Facilitating Professional Growth", Paper Presented At The Annual Meeting Of The American Educational Research Association, Montreal.

11/4/2012

Study of Students Health via Injuries which effect on their Absences in school

M. Zare (PhD) [1], A. Bahonar (PhD) [2], S. Alikhani (MD-MPh) [3], SH. Zakiani (MSc-PhD Student) [4], A. Zare (BSc) [5]

[1.] Research and Technology Deputy, Ministry of Health and Medical Education, Tehran, Iran
[2.] Faculty of Tehran University of Medical Science, Tehran, Iran
[3.] Ministry of Health and Medical Education, Tehran, Iran
[4.] Research and Technology Deputy, Ministry of Health and Medical Education, Tehran, Iran
zakiani2004@gmail.com
[5.] Faculty of Petroleum Engineering, Amirkabir University of Technology, Tehran, Iran

Abstract: Background: Study on the prevalence and pattern of the occurrence of injuries on school children and its effect on the health and education of students is considered very important. Material and Methods: 2003 school children from the elementary, guidance and high school in Pakshar were recruited in a cross-sectional study with cluster sampling method. Health Behavior in School-aged Children (HBSC) was used as questionnaire in survey. Results: 44.2% of the subjects had a history of injury in the last 12 months with a higher prevalence in boys than in girls ($p<0/01$) and with more incidence of trauma in the lower age. 50.4% of the total injuries have resulted to school absences with a mean of 6/16 days (SD=1/16) and 64.6% had absences of 3 days or less. Conclusion: Education on injury prevention in schools especially among the boys is necessary and attention must be given more to children on the lower age.

[M. Zare, A. Bahonar, S. Alikhani, SH. Zakiani, A. Zare. **Study of Students Health via Injuries which effect on their Absences in school.** *Life Sci J* 2012;9(4):4779-4782] (ISSN:1097-8135). http://www.lifesciencesite.com. 718

Keywords: Health; Absence of school; Accident; Cross-sectional; Injury; students; Trauma

1. Introduction

Events leading to injuries are the fundamental problem of the modern world and are the leading cause of death, hospitalization and disabilities in all age groups from 1 to 45 in the United States [1]. 71% of all of the total deaths in this country in the ages ranging from 10-24 were due to four main causes namely: vehicular accidents, other unintentional injuries, suicide and murder [2]. These accidents in addition to injuries such as poisoning, burns, falls, gunshot wounds and the ingestion of foreign objects are the leading cause of children's death in such a way that these preventable deaths have resulted to 40-50% deaths in the early and middle childhood and 75% of adolescents' deaths. Annually, approximately 32 million American children die as a consequence to these incidents, thousands suffered injuries and others survive but are suffering permanent brain damage and physical disabilities [3]. These injuries, especially for students in an athletic training environment are of great importance and are significant. A study conducted in Canada have showed that per hundred of youth who participated in sports activities annually, 40.2% of these injuries need to be seen by a doctor and 8.1% of these injuries needs urgent attention [4]. A cohort study conducted in America has found that 9032 high school students from 1995 to 1997 who actively performed physical exercises have been reported to suffer injuries of which 23.4% were accounted to the boy's football while the injuries for girl's football totaled to 26.7% [5]. Studies have shown that more than 50% of injuries obtained from sprains and dislocations and other injuries requiring surgery as well have been mainly related to the knees [6]. On the student's side, more academic and social skills must be gained for their future lives. Students' excessive absences could lead to educational failure especially if this group of students will have more than 11% of absences from the total number of school days. Annually, 1-3 students will be referred to the emergency department due to minor injuries but its impact on the school attendance was not given much attention. Therefore we could conclude that minor injuries do not require any hospitalization and this has no effect on the ability and mobility of the students to care for themselves [7]. Also a study conducted by Hyman and associates in 2007 have shown that an injured student's social and economic background has its impact on the school attendance and injuries have significant impact on the students' social status and school performance and on their family as well[8,9]. A study conducted in Yazd in the year 2000 have shown that 24% of injuries existing in the society are individuals less than 20 years belonging to the age groups of 7-20 years old, children and adolescents living in the city and the male sex are more vulnerable to various injuries [10]. Considering the social and economic consequences brought about by injuries and the importance of giving more attention to school children and adolescent students for the reason that the majority of

the population is composed of this group, this study was conducted for the purpose of evaluating the prevalence of the types of injuries and their effect on the students' absences in Pakdasht schools.

2. Methods

This cross-sectional study was implemented on the school children from the fifth grade (11years), guidance school (13 years) and first year high school (15years) respectively and based on international research standards, a study on the health behaviors of school age children in the city of Pakdahst was conducted. In order to fulfill of Health Behavior in School-aged Children (HBSC) questionnaire, a number of school health personnel of the health and treatment networks were trained to implement the educational programs. The cluster sampling was employed in such a manner that the list of school children in the 5th grade elementary, second year of the guidance school and the first year high school students of the city were obtained and with the use of a random number table, the number of classes segregated by gender were selected. After coordinating with the Ministry of Education, the interviewers were present in the schools selected as pilot of this project and were responsible for the distribution of the questionnaire to the students and explain any questions that arise, then the questionnaire were completed by the students themselves. Data results were analyzed with the use of descriptive statistics (absolute and relative frequency of average and standard deviation) and inferential statistics (Chi-square tests, T-test and ANOVA), 95% confidence level of the statistical test was considered.

3. Results

The questionnaires were distributed among the students and 1,872 students responded to the questions (93.5%). During the 12 months prior to the study, 55.8% of the students did not obtain any injuries and 12.9%, 9, 5.3% and 5.1% have obtained injuries for once or four times respectively. The frequency of injury in boys was significantly higher than in girls (P<0.01). The higher the educational level of the students, the lesser the injuries obtained in such a manner that the 5th grade elementary and 2nd year guidance school students significantly obtained more injuries than the first year high school students (P<0.01) but between the 5th grade elementary and 2nd year guidance school students, no significant difference was noted (Table 1).

Table 1: Relative frequency of occurrence of different types of injuries that have resulted to students' treatment in Pakdasht city segregated by sex and educational level

Educational Level	5th grade elementary		2nd year guidance school		First year high school		Total	
Number of occurrence	Girls No=326	Boys No=309	Girls No=317	Boys No=311	Girls No=303	Boys No=306	Girls No=946	Boys No=926
None	58.6	39.2	63.4	46	74.3	53.6	65.2	46.2
Once	22.7	31.7	22.1	27.3	15.2	21.2	20.1	26.8
Twice	8.6	10.4	7.9	12.9	5.3	12.7	7.3	12
3times	4	9.1	4.4	6.1	3.3	7.2	3.9	7.5
4 times	6.1	9.7	2.2	7.7	2	5.2	3.5	7.6
Total	100	100	100	100	100	100	100	100

Approximately, 40% of the students' injuries occurred at home of which, in this ratio the girls significantly obtained more injuries than the boys (P<0.01) but boys obtained more injuries in school in comparison to the girls (P<0.01). Also sports related injuries in school and in the street and or those injuries obtained on their way to school are significantly higher among the boys than in girls (P<0.01) but with regards to other cases, the ratios were relatively closer (Table 2).

Regarding injuries that occurred during the performance of activities, the highest injuries were obtained during playing or sports activities and bicycling and these related injuries were significantly higher in boys than in girls (P<0.01) while in other activities, the girls significantly obtained more injuries than the boys (Table3).

Table 2: Relative frequency of location in the occurrence of injury among students in the city of Pakdasht segregated by sex

Sex Location of the occurrence of injury	Girls (Percent) No=303	Boys (Percent) No=468	Total (Percent) No=771
At home or in the backyard	50.8	30.3	38.4
School	15.2	20.5	18.4
Stadium or sports field	4.3	14.1	10.2
Street, road or parking	7.3	15.4	12.2
Business or workplace	2.3	2.4	2.3
Outside the city	3.6	4.1	3.9
Other highways	16.5	13.2	14.5

60% of the problems that occurred as a result to the injuries include: bone fractures, joint dislocations, sprains, muscle strains and wounds. 44.6% of these injuries were treated in the doctor's clinics while 11.6% of cases were treated in the emergency department and in this case, treatments

were significantly higher among the boys in comparison to the girls (Table 4).

Table 3: the relative frequency of activities that resulted to students injuries in the city of Pakdasht segregated by sex

Sex Activities that resulted to accident	Girls No= 304	Boys No=457	Total No=761
Bicycling	16.1	28.7	23.7
Playing or practicing a sport or recreational activity	17.7	28.9	24.5
Skating	6.3	3.7	4.7
Walking or running	11.8	8.8	10
Car driving or motor driving	1.3	3.7	2.8
Fight	7.9	8.5	8.3
Work or daily activities	0.3	2.4	1.6
Others	38.5	15.3	24.6

Table 4: Relative frequency on the location of treatment for injured students in the city of Pakdasht segregated by sex

sex place	Girls No=307	Boys No=466	Total No=773
Doctor's clinic	48.9	41.8	44.6
Clinic or health center	14.7	17.6	16.4
Hospital's emergency department	8.5	13.7	11.6
School clinic	2.6	5.4	4.3
Others	25.4	21.5	23

Regarding the rate of absences from school, data analysis have shown that injuries which have occurred in 371 of cases (50.4%) have resulted to school absences and 219 of cases that reported the number days being absent was an average of 6/16 days(6/10±). 4.64% of this cases have resulted 3days or lesser of school absences. The higher the educational attainment, the days of absences acquired due to injury rises, although the one way ANOVA showed no significant difference.

4. Discussion

The overall rate of injuries among the boys were higher in comparison to the girls and the main reason for this is due to over activity and the intrinsic nature of this gender to be hyperactive as shown in our study and in the researchers conducted by others [10-15]. In similar studies, lower educational level has shown more risk of injuries [10-12]. In the United States in the year 1995 and 1997, 50% of the pattern of problems incurred as a result of injuries especially on cases of dislocation, sprains, and muscle strains were accounted to high school students while dislocation and sprains were among the first 5 injuries among the students [4]. A study conducted by Duggan entitled "A Profile of Injuries Occurring at a Rural Primary School in North East Victoria, the pattern of injury at school has been analyzed according to gender and on the following criteria: the proportion of injuries occurring in the different age groups, the time of day, week an year when injuries seem to peak, the body part injured, the cause of the injury and the nature of the injury. In this study, the incidence of injury in boys was higher than in girls. The incidence of injury between boys than girls was recorded in the ratio of 1.7to1. From the 28 injuries recorded, 63% of the boys and89% of all girls injured had subsequent absences from school. Also, by comparison, the different pattern of injuries include: 22% head, 51% upper extremities, 21% lower extremities and 6% for others [16].

Also the result of a study conducted among 4-14 years old Irish students has shown that, 75.4% of the injuries involved the lower extremities while 24.5% involved the upper extremity. The injury that caused more absences on the students was fracture and the average duration of absences from school was determined to be 3 days [17].

Another study conducted by Sosnowska and et.al. in 2003 entitled "Epidemiology of school accidents during a six school-year period in one region in Poland" showed that from the total of 50,000 students with the age range of 7-15, the total number of school accidents per 293,000 students-years was 3274 and the pattern of the occurrence of injuries were in the following manner: 36, 6% occurred during recess, and 33.2% during physical education which were most common as shown in this study. This study has also showed on how strategic planning must be employed in order to reduce school accidents [18].

Our study has shown that of the total population under study, 18, 4 % of the injuries happened within the school premises of which the incidence were higher in the boys (20.5%) in comparison to the girls (15.2%) table No. 2.

A study conducted on Chinese students has also showed that 21.7% injuries that have occurred on the boys and 17.6 % of the injuries that has occurred on the girls were in the school premises which are estimated to be close to our findings [11]. The prevalence of 12.2 % injuries that occurred in the roads and streets showed that the importance of this issue in our country must be considered a high priority. Problems and traffic injuries has been considered to be the leading cause of deaths in recent years. Studies have shown that a significant proportion of motor vehicle crashes are on pedestrians, shown as an example is on the age group ranging from 5 to 9 years old having the majority of proportion to vehicular accidents [19] and presents the highest rate of injury and mortality for both gender. The rate of traffic accidents incurred by individuals with an age range from 15 -19 are

doubled in comparison to individuals aged 18 and above.

As a whole, the overall incidence of injury to school children under study and the high rate of absences as a result to these injuries, necessitate awareness for more scientific studies in other regions of the country and to develop action plans for accident prevention through educational programs for students, teachers and families as well.

Corresponding Author:
SH. Zakiani (MSc-PhD Student)
Research and Technology Deputy
Ministry of Health and Medical Education
Tehran, Iran
E-mail: zakiani2004@gmail.com

References

1. Robertson LS. Injury control: Some effects, principles and prospects. In: oxford textbook of public Health Vol. 3,3rd ed. Oxford UN. Press Inc. New York, NY; 1997:1307-19
2. Eaton DK, Kann L, Kinchen S, Ross J, Hawkins J, Harris WA, et al. Youth risk behavior surveillance-United States 2005. MMWR 2006;55(5):1-108
3. Kronenfeld JJ, Glik DC. Unintentional injury: a major health problem for young children and youth. Journal of family and economic issues. 1995; 16:365-393.
4. Emery CA, Meeuwisse WH, McAllister JR. Survey of sport participation and sport injury in Calgary and area high schools. Clin. J Sport Med. 2006; 16(1):20-26.
5. Powell JW, Barber-Foss KD. Sex-related injury patterns among selected high school sports. Am J Sports Med. 2000 ;28(3):385-91
6. Powell JW, Barber-Foss KD. Injury Patterns in Selected High School Sports: A Review of the 1995-1997 Seasons. J Athl Train. 1999; 34(3):277-284.
7. Barnes MP, et al. Unnecessary school absence after minor injury: case-control study. BMJ. 2001; 323(7320): 1034–1035.
8. Hyman JE, et al. Risk factors for school absence after acute orthopaedic injury in New York City. J Pediatr Orthop. 2007; 27(4):415-20.
9. Sørensen L, et al .Sports injuries in school-aged children. A study of traumatologic and socioeconomic outcome. Scand J Med Sci Sports. 1998 Feb; 8(1):52-6.
10. Mazlomi seyed said, Hossein Fallahzadeh. Prevalence of accidents in people under 20 years old of Yazd. Payesh Journal. 2002 ;(4):21-25.
11. Ye-Huan S, Ignatius TY, Yan Z, Ya-Ping F, Shu-Qin G, Tze WW. Unintentional injuries among primary and middle school students in Maanshan City, eastern China. Acta Paediaterica. 2006;95: 266-273
12. Jia C, Zhao Z,Bo Q, Zhang Y, Liu Z. Incidence rates and risk factors for injury events in high school students in Lijin County, China. J Safety Res. 2005; 36:133-7.
13. Chen G, Smith GA, Deng S, Hostetler SG, Xiang H. Nonfatal injuries among middle-school and high-school students in Guangxi,China. Am J Public Health. 2005; 95:1989-95.
14. Sun YH, Yu IT,Wong TW,Zhang Y,Fan YP, Guo SQ. Unintentional injuries at school in China--patterns and risk factors. Accid Anal Prev. 2006;38:208-14
15. Lenaway DD, Ambler AG, Beaudoin DE. The epidemiology of school-related injuries: new perspectives. Am J Prev Med. 1992; 8:193-8.
16. Duggan N. School Injuries: A Profile of Injuries Occurring At A Rural Primary School In North East Victoria, 3rd National Rural Health Conference. Glenrowan, Victoria. 3-5 February 1995.
17. O'Farrell DA, et al. An epidemic of roller-blade injuries in children. Injury. 1997; 28(5-6):377-379.
18. Sosnowska S. T Kostka .Epidemiology of school accidents during a six school-year period in one region in Poland. Medicine European Journal of Epidemiology. 2003;18(10): 977-982
19. Rivara FP, Brownstein DR: Injury control, In: Nelson Textbook of pediatrics .15th ed .W.B.SAUNDERS CO.CH; 1998:226-32.
20. Sadeghi H A. Azizi F, Hatami H, Janghorban M. Epidemiology and Control of Common Disorders in Iran. Eshtiyagh press, Tehran; 2001:279-311.

11/6/2012

Natural Associations between Symbionts *Photorhabdus Spp.* and *Xenorhabdus spp.* and Bacteria Related to *Ochrobactrum anthropi, Bacillus pumilus and Enterobacter cloacae*

[1]Hussien A. Abouelhag and [2]Hanan A. El-Sadawy

[1] Microbiology and Immunology Department, National Research Centre, Dokki, Egypt
[2] Parasitology and animal diseases Department, National Research Centre, Dokki, Egypt
drabouelhag@yahoo.com

Abstract: *Xenorhabdus spp.* and *Photorhabdus spp.* are symbiotic bacteria that produce numerous metabolites of insecticidal and bactericidal impact. It was thought that these biological metabolites gave them the privilege of predominance in the gut of entomopathogenic nematodes (EPNs). The aim of this work was to investigate and characterize the associated bacteria with symbiotic bacteria *Photorhabdus* and *Xenorhabdus in-vitro* culture of EPNs (*Heterorhabditis indica* RM1, *Heterorhabditis sp.* S1 and *Steinernema abbasi*). The gut flora of EPNs were isolated and characterized morphologically and biochemically. The isolated associated strains were completely identified by restriction fragment length polymorphism and sequence analyses of PCR-amplified 16S rRNA. The isolated strains were *Ochrobactrum anthropi* and *Bacillus pumilus* associated with *Photorhabdus luminescens akhurstii* in the gut of *H. indica* RM1 and *Heterorhabditis sp.* S1 (Egyptian isolates). Also *Enterobacter cloacae* were associated with *X. indica* in the gut of *S. abbasi* (Omani isolate). These associated isolates are raising the concern about their effect in epidemiological maps in Egypt, Oman and their significance as public health threats. Regarding the common use of (EPNs), previous clinical case reports for these associated isolates and their developing record in human infectivity we should pronounce alarm of possible biological hazard of intensive use of EPNs without monitoring the hidden associated bacteria during their manufacture.

[Hussien A. Abouelhag and Hanan A. El-Sadawy. **Natural Associations between Symbionts *Photorhabdus Spp.* and *Xenorhabdus spp.* and Bacteria Related to *Ochrobactrum anthropi, Bacillus pumilus and Enterobacter cloacae***. *Life Sci J* 2012;9(4):4783-4790] (ISSN:1097-8135). http://www.lifesciencesite.com. 719

Keywords: *Ochrobactrum* spp., *Bacillus pumilus, Enterobacter cloacae Photorhabdus luminescens akhurstii*, *Xenorhabdus indica, Heterorhabditis indica*, *Steinernema abbasi,* 16S rRNA Gene Polymorphism and Sequencing.

1. Introduction

Entomopathogenic nematodes (EPNs) are biological control agents that are pathogenic to a wide range of insect pests **(*Grewal et al.*, 2005)**. Bacteria of the genera *Xenorhabdus* and *Photorhabdus* are known to be symbiotically associated with the soil dwelling EPNs of the family *Steinernematidae* and *Heterorhabditidae*, respectively **(*Marokhazi et al.*, 2004)**.

It has been assumed that the monoxenic association between the nematode and its symbiont is referred to bactericidal compounds produced by the symbiont during the reproduction of the nematode in the insect. All *Steinernema* spp. and *Heterorhabditis* spp. carry in their gut symbiotic bacteria of the genus *Xenorhabdus* **(*Thomas* and *Poinar,* 1979*; Akhurst* and *Boemare, 1988*)** and genus *Photorhabdus* **(*Boemare et al.*, 1993; *Akhurst et al.*, 1996; *Fischer-Le Saux et al.*, 1999) respectively**. These bactericidal compounds are believed to avert the development of other bacteria in the insect cadaver. However, several reports have shown that there are infrequently bacteria other than the unique symbiotic bacteria in the gut of nematodes. ***Lysenko*** and ***Weise*** **(1974)** isolated bacteria such as *Alcaligenes*, *Pseudomonas* and *Acinetobacter spp.* from *Steinernema carpocapsae*. Also ***Boemare*** **(1983)** isolated other associated bacteria such as *Pseudomonas aureofaciens*, *Pseudomonas fluorescens*, *Enterobacter agglomerans* and *Serratia liquefaciens* from *S. carpocapsae*. Similar observations were reported for *Steinernema scapterisci*, which was transferred from South America and sub cultured many times in Florida. This nematode was associated with *Ochrobactrum anthropi*, *Paracoccus denitricans*, *Pseudomonas maltophilia* and *Xenorhabdus spp.* **(*Aguillera* and *Smart,* 1993; *Aguillera et al.*, 1993).** It is noteworthy to mention that ***Babic et al.*** **(2000)** studied the naturally occurring bacteria isolated in association with *Photorhabdus luminescens* from tropical *Heterorhabditis spp.* in the Caribbean basin by using conventional phenotypic tests, restriction fragment length polymorphism and sequence analyses of PCR - amplified 16S rRNA genes (16S rDNAs) and they recorded the isolation of bacteria characterized as *Ochrobactrum* spp. and associated with the natural symbiont *Photorhabdus luminescens* subsp. *akhurstii* in the entomopathogenic nematode *Hterorhabditis indica* from the Caribbean basin. Some of these associated bacteria are of great benefit and in complete harmony with *Xenorhabdus, Photorhabdus* spp. bacteria as many researchers

showed a great similarity in many biological metabolites as proteases (zinc metaloproteases), this similarity is not restricted only to chemical nature (***Bowen et al.,* 2003**) but also extended to include the genes coding for such enzyme as many scientists proved that there is many sharing genes coding for enzymes responsible for hemolysis of sheep RBCs (hemolysis similarity between *P. luminescens* and *Serratia marcescens*) (***Brillard et al*., 2002**).

Actually the similarity between *Xenorhabdus-Photorhabdus* with associated bacteria not restricted only to similarity in bacterial enzymes it also extended to the similarity of bacterial toxins **(*Hertle,* 2005**) which plays a significant role in the control and destruction of insect host and their toxicity through secretion of these toxins in insect hemolymph as what occurs with *Serratia marcescens* which plays main role in inducing of infectivity in *Galleria mellonella* larvae by *S. carpocapsae filipjev* (***Ortega-Estrada et al.,* 2012).** This phenomena was also reported by ***Abebe et al.* (2011)** where *Serratia sp*. was able to kill *G. mellonella* in a manner similar to that induced by *Heterorhabditis* and *Photorhabdus* species. ***Sundra et al.* (1993**) postulated that *X. nematophila* indole antibiotics act as regulator to one of the associated bacteria (*P. putida*) and the upper hand seems to be for *Xenorhabdus* bacteria.

Bacillus spp. N. strain associated with *Rhabditidis* (EPNs) bacteria produces bioactive compounds (antibacterial and fungicidal) **(*Kumar et al.,* 2012b)** and these associated bacteria was isolated from 3rd stage infected juveniles of the nematode sample collected from hemolymph of *Galleria mellonella* (***Kumar et al.,* 2012a**). Also *Serratia sp.* showed a similar action against *G. mellonella* (***Abebe et al., 2011***).

The harmony between *Xenorhabdus, Photorhabdus* spp. and associated bacteria not only restricted to production of similar enzymes or carrying the same coding genes or even in synergistic mode of action for controlling infectivity of insects but many scientists extended their work to prove the evolutionary relationship between *Xenorhabdus, Photorhabdus spp.* and their associated bacteria where ***Moran et al.* (2005)** proved the evolutionary relationship between *Serratia spp.* and *Photorhabdus* spp. by phylogenic analysis of partial sequences of genes by 16S rRNA gene sequence.

The apprehension issue here is the emphasis of the biohazard activity of some of these associated bacteria especially that some strains like *E. faecalis* produced cytolysin toxin that causes urinary tract infection, bacteremia and endocarditis (***Fieldhouse et al.,* 2010)** like what reported for *Photorhabdus lumensense* and *Photorhabdus asymbiotica* in North America and Australia by ***Peel et al. (1999)***.

It must be kept in mind that *Xenorhabdus* and *Photorhabdus* are used intensely in commercial products for biological control of agricultural insects. So, great consideration must be given for the bacterial load carried by entomopathogenic nematodes *in-vitro* culture. This research targeted exploration of bacteria that present in association with *Photorhabdus luminescens akhurstii* isolated from EPNs *Heterorhabditis indica* RM1 and *Heterorhaditis* sp. S1 isolated from Egyptian soil Also *Xenorhabdus indica* isolated from the Omani EPNs *S. abbasi*.

2. Materials and Methods

Nematode propagation

Heterorhabditis indica RM1 and *Heterorhaditis* sp. S1 are entomopathogenic nematodes (EPNs) isolated from Egyptian soil while *Steinernema abbasi* Ab nematode was isolated from Omani soil. They were maintained in the laboratory by several passages through last-instars larvae of *Galleria mellonella* according to ***Dutky et al.* (1964)** in the Department of Parasitology and Animal Diseases, National Research Centre, Dokki, Egypt.

Bacterial isolates

The origins of the bacterial strains were listed in table 1. *Xenorhabdus nematophila* 19061, and *Photorhabdus luminescens* TT01 as standard strains were obtained from University of Wisconsin, Milwaukee. The bacteria associated with infective juveniles were isolated by the hanging-drop technique **(*Poinar* and *Thomas,* 1966)**. A sterile drop of insect hemolymph was collected after surface sterilization of infected *Galleria mellonella* (10 min in 1% sodium hypochlorite solution) and rinsed three times in sterile water. The microorganisms developing in the drop after 48 h at 26 °C were streaked on MacConkey agar. Unknown bacteria associated with *Photorhabdus luminescens akhurstii* RM1, S1 and *Xenorhabdus indica* Ab phase I, which was found in reared *Heterorhabditis* and *Steinernema* nematodes were repeatedly isolated on this medium. These *Photorhabdus luminescens* - associated strains were designated as SIA and RMIA while those associated with *Xenorhabdus indica* were designated as AbA.

Culture conditions

The optimal growth temperature was determined by streaking each isolate on NBTA medium (nutrient agar supplemented with 25 mg bromothymol blue l^{-1} and 40 mg triphenyltetrazolium chloride l^{-1}) and incubating at 26 °C. All the *Photorhabdus* and *Xenorhabdus*-associated isolates grew better at 26 °C, and consequently all the biochemical tests described below were conducted at this temperature. Isolates were grown in Luria broth (LB) (Difco- Fisher Scientific) on a shaking rack for 24 h. The *Photorhabdus* and *Xenorhabdus*- associated isolates were maintained on nutrient agar plates at

5 °C. *Xenorhabdus nematophila* 19061 and *Photorhabdus luminescens* TT01 strains are usually cultivated at 26 °C. They were maintained on NBTA medium at 15°C **(*Akhurst,* 1980)**.

Phenotypic characterization of the *Photorhabdus* and *Xenorhabdus*- associated isolates.

Phenotypic characterization was conducted according to ***Bergey's Manual* (*Holt et al.,* 1994)**, and later reports **(*Alnor et al.,* 1994** and ***Velasco et al.,* 1998)** and additional tests summarized below. Colonial morphology was observed on nutrient agar and the diameter of colonies was measured after 24 and 48h in five independent experiments. Dye adsorption on MacConkey agar or NBTA, and the test for bioluminescence were conducted according to ***Boemare*** and ***Akhurst* (1988)**. The cell wall was characterized by the Gram stain test **(*Cerny,* 1976)**. The biochemical reactions were carried out according to ***Holt et al.,* 1994** and they included the following: catalase reaction, oxidase reaction, sugar fermentation (lactose, sucrose, esculine), urease reaction, growth on bile agar, nitrate reduction test, indol reaction, MRVP reaction. Haemolysis was determined on Tryptic Soy Agar (BioMerieux) supplemented with 10 % (v/v) sterile defibrinated sheep blood (BioMerieux).

DNA extraction and PCR amplification of 16S rRNA genes

DNA extraction and PCR amplification of 16S rRNA genes was carried out according to ***Van der Hoeven et al.* (2008)**. Based on morphological and Gram stain results, bacterial isolates were chosen for amplification of 16S rRNA genes. In most cases, PCR was carried out using a direct colony PCR approach. In cases where a product was not obtained, DNA was extracted from 2-mL LB overnight cultures of individual isolates grown at 26 °C using the Edge BioSystems Bacterial Genomic DNA Purification Kit. The universal bacterial primers 50-GTTTGATCCTGGCTCAG-30 (11F) and 5´-ACGGYTACCTTGTTACGACTT-3´ (1512R) were used in the PCR reaction. A small amount of cells was scraped from the colony using a sterile pipette tip, resuspended in 3 mL distilled PCR-grade water and boiled for 5 min. The boiled cells were added to 47 µl of a PCR mixture containing 2 mM forward and reverse primers, 2.5 mM $MgCl_2$, 200 mM dNTPs, 5µl $MgCl_2$-free 10X Taq polymerase buffer and 1.25 U Taq DNA polymerase (Promega Co.). PCR amplification was carried out as follows: 30 s denaturing at 94 °C, 30 s annealing at 55 °C and 1-min extension at 72 °C for 30 cycles. PCR products were purified using the High Pure PCR Product Purification Kit (Roche) and analyzed on 0.7% agarose gels (Ref.??).

Nucleotide sequence analysis of purified PCR products

Nucleotide sequence analysis of purified PCR products was performed at the University of Chicago, Cancer Research Center, DNA Sequencing and Genotyping Facility. BLASTN search of the NCBI database was used for genus designation with sequences of length c. 760 bp. To further confirm identification and grouping of genera, sequences were aligned with CLUSTALW and compared with the sequences in Ribosomal Database Project II (RDP-II), using the Sequence Match function (http://rdp.cme.msu.edu/seqmatch/seqmatch_intro.jsp). In all cases, 16S rRNA gene analysis was consistent with microbiological data.

3. Results

The isolated strains from *Heterorhabditis indica* RM1 and *Heterorhabditis* spp. S1 which are usually engaged with *Photorhabdus luminescens akhurstii* as a symbiotic bacteria was unexpectedly associated with another Gram negative small bacilli designated as SIA and RMIA (Table 1) which gave red coloration and different biochemical reaction as illustrated in table 2.

Also, another bacterial strain was isolated from *Steinernema abbasi* Ab which is usually occupied with *Xenorhabdus indica*. This Gram negative small bacillus was designated as AbA (Table 1). It gave red color colony on NBTA agar and different biochemical reaction as illustrated in table 2.

Table 1: *Photorhabdus* and *Xenorhabdus* - associated isolates and laboratory strains used in this study

Insect Host	Entomopathogenic nematodes (EPNs)	Symbiotic bacteria	Associated isolates
Galleria melonella	*Heterorhabditis indica* RM1	*Photorhabdus luminescens akhurstii*	**RMIA**
	Heterorhabditis sp. S1		**SIA**
	Steinernema abbasi Ab	*Xenorhabdus indica*	**AbA**
	Steinernema carpocapsi	*Xenorhabdus nematophila* 19061	**Standard laboratory strains from University of Wisconsin, Milwaukee, USA**
	Heterorhabditis indica	*Photorhabdus luminescens* TT01	

The biochemical reactions of strain SIA were summarized in table 2 and the most prominent results were recapitulated in the lacking of luminous character of genus *Photorhabdus*, weak positive catalase reaction, incapability of sugar fermentation (sucrose, lactose and esculin), positive urease reaction and loss of capability to grow on bile agar.

The biochemical reactions of strain RMIA as summarized in table 2 showed remarkable lacking of luminous character of genus *Photorhabdus*, positive

catalase reaction, positive sugar fermentation (sucrose, lactose and esculin), positive VP reaction and had the capability to grow on bile agar (table 2).

Table 2: Phenotypic characters distinguishing *Photorhabdus* and *Xenorhabdus*-associated isolates.

Phenotypic character	*P. luminescens TT01*	*X. nematophila 19061*	*AbA*	*SIA*	*RMIA*
Colony color on NBTA agar	Blue	Blue	Red	Red	Red
Luminescent	[+ve]	-ve	-ve	-ve	-ve
Gram's stain	-ve	-ve	-ve	-ve	-ve
Shape	Rods	Rods	Rods	Rods	Rods
Motility	Motile	Motile	Motile	Motile	Motile
Catalase reaction	-ve	-ve	[+ve]	[+ve] W	[+ve]
Oxidase reaction	-ve	-ve	-ve	[+ve] W	-ve
Sugar fermentation:					
lactose	[+ve] W	[+ve] W	[+ve] W	-ve	[+ve]
Sucrose	[+ve] W	[+ve] W	[+ve] S	-ve	[+ve] S
Esculin	[+ve] W	-ve	-ve	-ve	[+ve] S
Urease reaction	-ve	-ve	-ve	[+ve]	-ve
Growth on bile agar	[+ve]	-ve	-ve	-ve	[+ve]
Nitrate reduction test	-ve	-ve	[+ve]	-ve	-ve
Indole reaction	-ve	-ve	-ve	-ve	-ve
MR reaction.	-ve	-ve	-ve	-ve	-ve
VP reaction	-ve	-ve	[+ve] ^{W}	-ve	[+ve] ^{S}
Hemolysis	-ve	[+ve]	-ve	-ve	-ve

All tests were done at 37°C, weak positive [+ve] ^{W}, strong positive, [+ve] ^{S}, positive [+ve], negative -ve. The most discriminatory characters are shown in bold.

The biochemical reactions of AbA were characterized by positive catalase reaction, positive nitrate reduction test, and positive VP reaction and the rest of the reactions were similar to *X. nematophila 19061* except colony color and hemolysis characters as shown in table 2.

Bacterial identification by 16SrRNA

The identification of the isolated strains were confirmed by using specific PCR probe using specific primers where the bacterial PCR products (1.4 kbp) were screened using 0.7 agarose gel electrophoresis (Fig. 1).

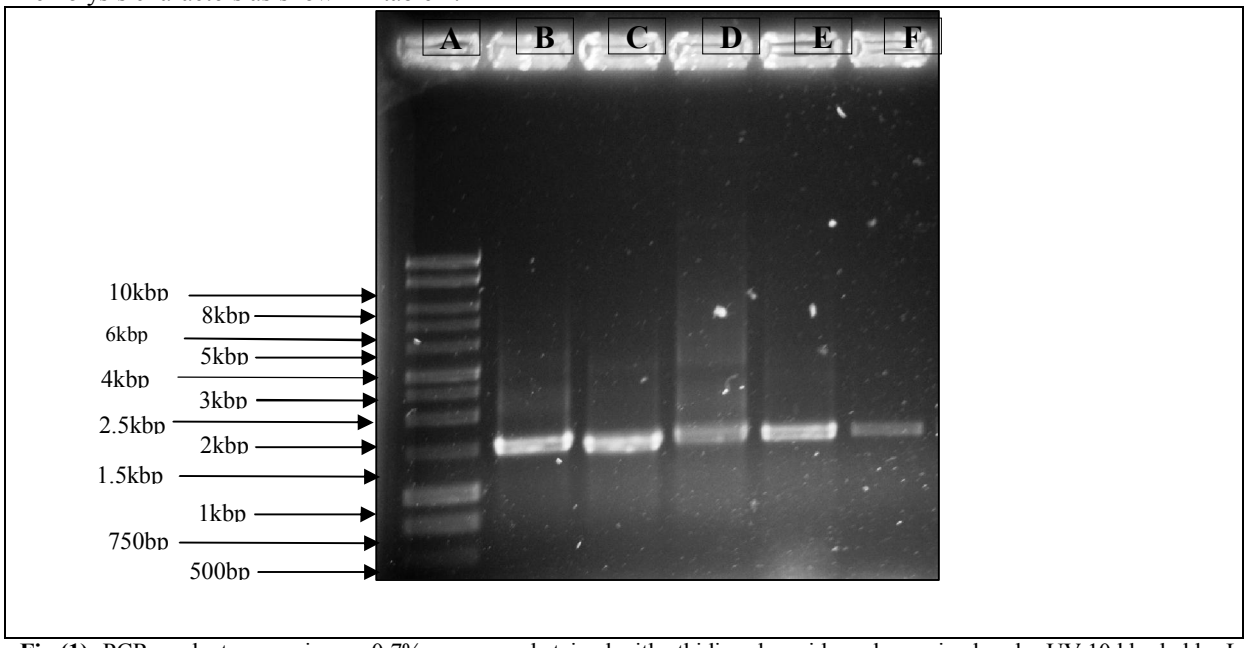


Fig (1): PCR products screening on 0.7% agarose gel stained with ethidium bromide and examined under UV 10 kbp ladder Lane (A), *P. luminescens* (TT01) Lane (B), *X. nematophila* (19061) lane (C), *AbA* isolate Lane (D), *SIA* isolate Lane (E) and *RMIA* isolate Lane (F).

Nucleotide sequence analysis of purified PCR products.

The Nucleotide sequence analysis of purified PCR products (Done at Chicago Cancer Research Center) and the BLASTN search of the NCBI database resulted in identification of isolate SIA as *Ochrobactrum anthropi*, isolate RMIA was defined as *Bacillus pumilus,* while isolate AbA was defined as *Enterobacter cloacae*.

For further confirm identification and grouping of genera, sequences were aligned with CLUSTALW and compared with the sequences in Ribosomal Database Project II (RDP-II), using the Sequence Match function (http://rdp.cme.msu.edu/seqmatch/ seqmatch_intro.jsp).

Table 3: Nucleotide sequence analysis of purified PCR products

Designated Code	Defined strain	Max score	Total score	Query coverage %	Error value	Max ident. %
SIA	*Ochrobactrum anthropi* strain SRK5	**1881**	1881	100	0.0	99
RMIA	*Bacillus pumilus* strain CTSP14	**1482**	1482	100	0.0	99
AbA	*Enterobacter cloacae* isolate	**1796**	1796	100	0.0	100

4. Discussion

The present work reported the isolation of three strains according to 16sRNA plast. These strains are *Ochrobactrum anthropi*, *Bacillus pumilus* and *Enterobacter cloacae*. These strains were associated with the symbiotic bacteria as they were isolated from the infected insect (*Galleria mellonella*) hemolymph. *Ochrobactrum anthropi* and *Bacillus pumilus* were associated with *Photorhabdus luminescens akhurstii* while *E. cloacae* was associated with *Xenorhabdus indica*.

The sterilization technique followed during the process of hemolymph collection from infected insect is well established by many authors in this field (***Razia et al., 2011***) and the frequency of isolation gave no doubt in any possibility of contamination.

Some of the isolated associated strains like *O. anthropi* had been recognized and mentioned in previous reports (***Razia et al.,* 2011**) while according to our knowledge *B. pumilus* and *E. cloacae* was not reported previously in association with symbiotic bacteria. The main concern here is the health hazard significance of these associated bacteria with the symbiotic bacteria (*Xenorhabdus* and *Photorhabdus* sp.) **(*Babic et al.,* 2000)**. The contribution of symbiotic bacteria in *in-vitro* culture of entomopathogenic nematodes as biological pesticides is well established and well spread all over the world. It was believed that the antimicrobial toxins and metabolites of symbiotic bacteria gave no chance for growth of any other microorganisms but these recent reports smashed out this theory and raised an urgent query about the pathogenicity and the infectivity of such associated bacteria. In order to estimate the bio safety of these associated bacteria we should appraise the nature of isolated strains and the previous clinical case reports interrelated with them.

Ochrobactrum anthropi is a Gram-negative, aerobic bacillus previously classified by Center of Disease Control and prevention (CDC) as group Vd. *O. anthropi* is motile organism characterized by production of urease and oxidase (***Holmes et al.,* 1988; *Bruckner*** and ***Colonna.* 1993),** it is wide spread ecologically in nature including soil and water and contaminated pharmaceuticals (***Barson et al.,* 1987**; ***Holmes et al.,* 1988**; ***Deliere et al.,*** 2000; ***Peltroche-Llacsahuanga et al.,* 2000)**. *Ochrobactrum anthropi* had been isolated from different human clinical sources as blood, urine, wounds, feces and oral and vaginal secretions, leading to its specific epithet anthropi **(*Holmes et al.,* 1988)**.

Ochrobactrum anthropi is believed to be an opportunistic organism that can affect mainly immune compromised individuals and indwelling catheters **(*Vaidya et al.,* 2006)** and the first record of clinical infection with *O. anthropi* was recorded at 1980 in debilitated patients with pancreatic abscesses **(*Appelbaum and Campbell* 1980).** However lately many clinical cases reported affections with *O. anthropi* in healthy individuals which confirm their ability to cause infection **(*Mahmood, et al.,* 2000*; Vaidya et al.,* 2006; *Alparslan et al.,* 2012; *Quintela Obregón et al.,* 2012).** There are many evident reports supporting this finding as *O. anthropi* caused endophthalmitis **(*Chiang et al.,* 2009** and ***Song et al.,* 2007)**, osteomyelitis **(*Wheen et al.,* 2002)**, meningitis **(*Christenson et al.,* 1997)**, sepsis associated with an infected venous catheter **(*Wi and Peck* 2010)**, contaminated pharmaceuticals **(*Kettaneh, et al.,* 2003**. and ***Mahmood, et al.,* 2000)**, pelvic abscess **(*Vaidya et al.,* 2006)**, septic shock **(*Ozdemir et al.,* 2006)**, and peritonitis (***Alparslan et al.,* 2012**). So we can conclude that *O. anthropi* can affect healthy individuals as well as immune compromised patients. The other public health issue that we must pointed to is the developed resistance of *O. anthropi* to antibiotics such as beta-lactams, ampicillin-clavulanate, pipracellin-tazobactams, cefotaxime, ceftriaxone, and aztreonam **(*Cieslak et al.,* 1996)**.

Ochrobactrum anthropi has been isolated from different geographical regions like Caribbean islands (***Babic et al.,* 2000)** and France (***Romano et al.,* 2009**)

and many other places in the world and here in Egypt we reported the isolation of *O. anthropi* in association with the symbiotic bacteria *Photorhabdus luminescence akhurstii* from the native isolates of *Heterorhabditis indica* RM1.

The second isolated strain was *Bacillus pumilus* which gave the identical phenotypic characters of the known strain as mentioned by ***O'Donnell et al.* (1980)**. It is also, noteworthy to highlight the significance of the other associated bacteria isolated in this study like *B. pumilus*. Other reports mentioned that *Bacillus* N strain is isolated from novel *Rhabditid* entomopathogenic nematode in India and this strain shows antibacterial and fungicidal activity (***Kumar et al*., 2012 a & b)** so this associated bacteria may had a significant synergistic effect in controlling the biological environment in the gut of EPNs and their host insect.

Enterobacter cloacae was isolated as an associated organism with *Xenorhabdus indica* which reside from *Galleria mellonella* infected with *Stienernema abassi* Ab. The isolated strain showed identical phenotypic characters to identical strain of *E. cloacae* recorded by known authors like ***Wang et al.* (1989)**. *Enterobacter cloacae* is widely dispersed in nature. However it is less frequently reported as endemic infectious agent for human but outbreaks were reported due to lack of hygienic measures and sanitation **(*Eckmanns et al.*, 2000).** *Enterobacter cloacae* as a significant nosocomial infective agent was reported in water and even in newly born intensive care unit (CIU) **(*LaTuga et al.*, 2011; *Crémet et al.*, 2012)**. Also, *E. cloacae* recorded developed resistance to many antibiotics which gave threaten alarm for public health concern **(*Mezzatesta et al.*, 2012)**.

It can be conclude that our results are in agreement with ***Babic et al.*, 2000** and conflict with what claimed by ***Boemare et al.* (1996),** that monoxenic nematodes obtained from the combination of surface axenized eggs with the natural symbiont should be used for this purpose.

So this work recommends that the *in-vitro* culture of entomopathogenic nematodes for mass production purposes should be strictly controlled to prevent any contamination and to avoid epidemiological spreading of the pathogenic associated bacteria.

Corresponding author
Hussien A. Abouelhag
Microbiology and Immunology Department, National Research Centre, Dokki, Egypt
drabouelhag@yahoo.com

References

1. Abebe E., Abebe-Akele F., Morrison J., Cooper V. and Thomas W. K. (2011). An insect pathogenic symbiosis between a *Caenorhabditis* and *Serratia*. *Virulence*; 2(2):158-61.
2. Aguillera, M. M. and Smart, G. C. and Jr (1993). Development, reproduction, and pathogenicity of *Steinernema scapterisci* in monoxenic culture with different species of bacteria. *J. Invertebr. Pathol.* 62, 289 - 294.
3. Aguillera, M. M., Hodge, N. C., Stall, R. E. & Smart, G. C. and Jr (1993). Bacterial symbionts of *Steinernema scapterisci*. *J. Invertebr. Pathol.* 62, 68 - 72.
4. Akhurst, R. J. (1980). Morphological and functional dimorphism in *Xenorhabdus* spp., bacteria symbiotically associated with the insect pathogenic nematodes *Neoaplectana* and *Heterorhabditis*. *J. Gen. Microbiol.* 121, 303 - 309.
5. Akhurst, R. J. and Boemare, N. E. (1988). A numerical taxonomic study of the genus *Xenorhabdus* (*Enterobacteriaceae*) and proposed elevation of the subspecies of *X. nematophilus* to species. *J. Gen. Microbiol.* 134, 1835 - 1845.
6. Akhurst, R. J., Mourant, R. G., Baud, L. and Boemare, N. E. (1996). Phenotypic and DNA relatedness study between nematode symbionts and clinical strains of the genus *Photorhabdus* (*Enterobacteriaceae*). *Int. J. Syst. Bacteriol.* 46, 1034 - 1041.
7. Alnor, D., Frimodt-Moller, N., Espersen, F. and Frederiksen, W. (1994). Infections with the unusual human pathogens *Agrobacterium* species and *Ochrobactrum anthropi*. *Clin. Infect. Dis*. 18, 914 - 920.
8. Alparslan C., Yavascan O., Kose E., Sanlioglu P. and Aksu N. (2012) An Opportunistic Pathogen in a Peritoneal Dialysis Patient: *Ochrobactrum Anthropi*. *Indian J Pediatr*. [Epub ahead of print]
9. Appelbaum P. C. and Campbell D. B. (1980). Pancreatic abscess associated with *Achromobacter* group Vd biovar 1. *J. Clin. Microbiol.*; 12(2):282-3.
10. Babic I., Fischer-Le Saux M., Giraud E. and Boemare N. (2000). Occurrence of natural dixenic associations between the symbiont *Photorhabdus luminescens* and bacteria related to *Ochrobactrum* spp. in tropical entomopathogenic *Heterorhabditis* spp. (Nematoda, *Rhabditida*). *Microbiology*; 146 (Pt 3):709-18.
11. Barson, W. J., Cromer B. A., and Marcon M. J. (1987). Puncture wound osteochondritis of the foot caused by CDC group Vd. *J. Clin. Microbiol.* 25:2014–2016.
12. Boemare, N. E. (1983). Recherches sur les complexes neumatobacteuriens entomopathogenes: eUtude bacteUriologique, gnotobiologique et physiopathologique du mode d'action parasitaire de *Steinernema carpocapsae* Weiser (Rhabitida: *Steinernematidae*). The' se d'Etat (PhD thesis), Universite! Montpellier II.
13. Boemare, N. E. and Akhurst, R. J. (1988). Biochemical and physiological characterization of colony form variants in *Xenorhabdus* spp. (*Enterobacteriaceae*). *J. Gen. Microbiol.* 134, 751 - 761.

14. Boemare, N. E., Akhurst, R. J. and Mourant, R. G. (1993). DNA relatedness between *Xenorhabdus* spp. (*Enterobacteriaceae*), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. *Int. J. Syst. Bacteriol.* 43, 249 - 255.
15. Boemare, N. E., Laumond, C. and Maule, H. (1996). The nematode - bacterium complexes: biology, life cycle, and vertebrate safety. *Biocontrol Sci. Technol*., 6, 333 - 345.
16. Bowen D. J., Rocheleau T. A., Grutzmacher C. K., Meslet L., Valens M., Marble D., Dowling A., Ffrench-Constant R. and Blight M. A. (2003). Genetic and biochemical characterization of PrtA, an RTX-like metalloprotease from *Photorhabdus*. *Microbiology*; 149 (Pt 6):1581-91.
17. Brillard J., Duchaud E., Boemare N., Kunst F. and Givaudan A. (2002). The PhlA hemolysin from the entomopathogenic bacterium *Photorhabdus luminescens* belongs to the two-partner secretion family of hemolysins. *J. Bacteriol*. 184(14):3871-8.
18. Bruckner, D. A., and Colonna, P. (1993). Nomenclature for aerobic and facultative bacteria. *Clin. Infect. Dis.*, 16:598–605.
19. Chiang C. C., Tsai Y. Y., Lin J. M. and Chen W. L. (2009). Chronic endophthalmitis after cataract surgery secondary to *Ochrobactrum anthropi*. *Eye (Lond)*;23(5):1237-8.
20. Christenson J. C., Pavia A. T., Seskin K., Brockmeyer D., Korgenski E. K., Jenkins E., Pierce J. and Daly J. A. (1997). Meningitis due to *Ochrobactrum anthropi*: an emerging nosocomial pathogen. A report of 3 cases. *Pediatr Neurosurg*.;27(4):218-21.
21. Cerny, G. (1976) Method for distinction of Gram positive from Gram negative bacteria. *J. Appl. Microbiol.*, 3, 223 – 225.
22. Cieslak T. J., Drabick C. J. and Robb M. L. (1996). Pyogenic infections due to *Ochrobactrum anthropi*. *Clin. Infect. Dis.*; 22(5):845-7.
23. Crémet L., Bourigault C., Lepelletier D., Guillouzouic A., Juvin M. E., Reynaud A., Corvec S. and Caroff N. (2012). Nosocomial outbreak of carbapenem-resistant *Enterobacter cloacae* highlighting the interspecies transferability of the blaOXA-48 gene in the gut flora. *J. Antimicrob. Chemother.*; 67(4):1041-3.
24. Delière E., Vu-Thien H., Lévy V., Barquins S., Schlegel L., Bouvet A. (2000) Epidemiological investigation of *Ochrobactrum anthropi* strains isolated from a haematology unit. *J. Hosp. Infect.*; 44(3):173-8.
25. Dutky, S.R., Thompson, J. V., and Cantwell, G. E. (1964). A technique for the mass propagation of the DD-136 nematode. *Journal of Insect Pathology*. 6:417 – 422.
26. Eckmanns T.; Rath A.; Ruden H.; Gastmeier P. and Daschner F. (2000). Outbreak of *Enterobacter cloacae* Related to Understaffing, Overcrowding, and Poor Hygiene Practices. *Infection Control and Hospital Epidemiology*, Vol. 21, No. 5, pp. 305-308.
27. Fieldhouse R. J., Turgeon Z., White D. and Merrill A. R. (2010). Cholera- and anthrax-like toxins are among several new ADP-ribosyltransferases. *PLoS Comput. Biol.*;6(12):e1001029.
28. Fischer-Le Saux M., Viallard V., Brunel B., Normand P. and Boemare N. E. (1999). Polyphasic classification of the genus *Photorhabdus* and proposal of new taxa: *P. luminescens* subsp. *luminescens* subsp. nov., *P. luminescens* subsp. *akhurstii* subsp. nov., *P. luminescens* subsp. *laumondii* subsp. nov., *P. temperata* sp. nov., *P. temperata* subsp. *temperata* subsp. nov. and *P. asymbiotica* sp. nov. *Int. J. Syst. Bacteriol.*, 49 Pt 4:1645-56.
29. Grewal, P. S., Ehlers, R. U. and Shapirollan, D. I. (2005) Nematodes as Biocontrol Agents. Wallingford: *CABI Publishing*. p. 505.
30. Hertle R. (2005). The family of Serratia type pore forming toxins. *Curr. Protein Pept. Sci.* 6(4):313-25.
31. Holt, J. G., Krieg, N. R., Sneath, P. A., Staley, J. T. and Williams, S. T. (1994). Bergey's Manual of Determinative Bacteriology, 9th edn. Baltimore: Williams & Wilkins.
32. Holmes, B., Popoff M., Kiredjian M., and Kersters K. (1988). *Ochrobactrum anthropi* gen. nov., sp. nov. from human clinical specimens and previously known as group Vd. *Int. J. Syst. Bacteriol.* 38:406–416.
33. Kettaneh, A., Weill F. X., Poilane I., Fain O., Thomas M., Herrmann J. L., and Hocqueloux L. (2003). Septic shock caused by *Ochrobactrum anthropi* in an otherwise healthy host. *J. Clin. Microbiol.* 41:1339–1341.
34. Kumar S. N., Mohandas C, Siji J. V., Rajasekharan K. N. and Nambisan B. (2012a) Identification of antimicrobial compound, diketopiperazines, from a *Bacillus* sp. N strain associated with a *rhabditid* entomopathogenic nematode against major plant pathogenic fungi. *J. Appl. Microbiol.*; 113(4):914-24.
35. Kumar S. N., Siji J. V., Rajasekharan K. N., Nambisan B. and Mohandas C. (2012b). Bioactive stilbenes from a *Bacillus* sp. N strain associated with a novel *rhabditid* entomopathogenic nematode. *Lett. Appl. Microbiol.* ;54(5):410-7.
36. Larsen, N., Overbeek, R., Harrison, S., Searles, D. and Garrity, G. (1997). Bergey's Revision of the RDP Tree. In http:// www.cme.msu.edu/Bergeys}btcomments/bt9.pdf, pp. 49 - 50. Edited by *J. Garrity & S. Harrison.* Baltimore: Williams & Wilkins.
37. LaTuga M. S., Ellis J. C., Cotton C. M., Goldberg R. N., Wynn J. L., Jackson R. B. and Seed P. C. (2011). Beyond bacteria: a study of the enteric microbial consortium in extremely low birth weight infants. *PLoS One* ;6(12):e27858.
38. Lysenko, O. and Weiser, J. (1974). Bacteria associated with the nematode *Neoaplectana carpocapsae* and the pathogenicity of this complex for *Galleria mellonella* larvae. *J. Invertebr. Pathol.* 24, 332 - 336.
39. Mahmood, M. S., A. R. Sarwari, M. A. Khan, Z. Sophie, E. Khan, and S. Sami. (2000). Infective endocarditis and septic embolization with *Ochrobactrum anthropi*: case report and review of literature. *J. Infect.* 40:287–290.

40. Marokhazi, J., Lengyel, K., Pekar, S., Felfoldi, G., Patthy, A., Graf, L., Fodar, A. and Venekei, I. (2004). Comparison of proteolytic activities produced by entomopathogenic *Photorhabdus* bacteria: strain- and phase-dependent heterogeneity in composition and activity of four enzymes. *Appl. Environ. Microbiol.* 70, 7311–7320.
41. Mezzatesta M. L., Gona F. and Stefani S. (2012) *Enterobacter cloacae* complex: clinical impact and emerging antibiotic resistance. *Future Microbiol.*; 7(7):887-902.
42. Moran N. A., Russell J. A., Koga R. and Fukatsu T. (2005). Evolutionary relationships of three new species of *Enterobacteriaceae* living as symbionts of aphids and other insects. *Appl. Environ. Microbiol.*;71(6):3302-10.
43. O'Donnell A. G., NORRIS J. R., Berkeley R. C. W., Claus D., Kanek T., Logan N. A., and Nozak R. (1980). Characterization of *Bacillus subtilis*, *Bacillus pumilus*, *Bacillus licheniformis*, and *Bacillus amyloliquefaciens* by Pyrolysis Gas-Liquid Chromatography, Deoxyribonucleic Acid-Deoxyribonucleic Acid Hybridization, Biochemical Tests, and API Systems. *International Journal of Systematic Bacteriology*, p. 448-459.
44. Ortega-Estrada, M. De J., M. Cristina Del Rincón-Castro, Basurto-Ríos R., Toledo R. and Ibarra J. E. (2012). Phoresis between *Serratia marcescens* and *Steinernema carpocapsae* (*Rhabditida: Steinernematidae*) during Infection of *Galleria mellonella* (Lepidoptera: *Pyralidae*) Larvae. *Florida Entomologist*, 95(1):120-127. 2012.
45. Ozdemir D., Soypacaci Z., Sahin I., Bicik Z. and Sencan I. (2006). *Ochrobactrum anthropi* endocarditis and septic shock in a patient with no prosthetic valve or rheumatic heart disease: case report and review of the literature. *Jpn. J. Infect. Dis.*; 59(4):264-5.
46. Peel M. M., Alfredson D. A., Gerrard J. G., Davis J. M., Robson J. M., McDougall R. J., Scullie B. L. and Akhurst R. J. (1999). Isolation, identification, and molecular characterization of strains of *Photorhabdus luminescens* from infected humans in Australia. *J. Clin. Microbiol.*;37(11):3647-53.
47. Peltroche-Llacsahuanga, H., Brandenburg V., Riehl J., and Haase G. (2000). *Ochrobactrum anthropi* peritonitis in a CAPD patient. *J. Infect.* 40:299–301.
48. Poinar, G. O., Jr and Thomas, G. M. (1966). Signifcance of *Achromobacter nematophilus* Poinar and Thomas (Achromobacteriaceae: *Eubacteriales*) in the development of the nematode DD-136 (Neoaplectana sp., Steinernematidae). *Parasitology* 56, 385 - 390.
49. Quintela Obregón E., Palomar Fontanet R., Salas C., Rodrigo Calabia E. and Arias Rodríguez M. (2010). *Ochrobactrum anthropi* and polymicrobial peritonitis in peritoneal dialysis: a resistance predictor. Nefrologia.; 30(5):591-2.
50. Razia M, Karthikraja R, Padmanaban K, Chellapandi P, Sivaramakrishnan S. (2011). 16S rDNA-based phylogeny of non-symbiotic bacteria of Entorno-pathogenic nematodes from infected insect cadavers. *Genomics Proteomics Bioinformatics*.; 9 (3):104-12.
51. Romano S., Aujoulat F., Jumas-Bilak E., Masnou A., Jeannot J., Falsen E., Marchandin H. **and** Teyssier C. (2009). Multilocus sequence typing supports the hypothesis that Ochrobactrum anthropi displays a human-associated subpopulation. BMC Microbiology, 9:267.
52. Song S., Ahn J. K., Lee G. H. and Park Y. G. (2007). An epidemic of chronic pseudophakic endophthalmitis due to *Ochrobactrum anthropi*: clinical findings and managements of nine consecutive cases. *Ocul. Immunol. Inflamm.*;15(6):429-34.
53. Sundar, L., and Chang F. N. (1993). Antimicrobial activity and biosynthesis of indole antibiotics produced by *Xenorhabdus nematophilus*. *J. Gen. Microbiol.* 139:3139-3148.
54. Thomas, G. M. and Poinar, G. O., Jr (1979). *Xenorhabdus* gen. nov., a genus of entomopathogenic nematophilic bacteria of the family *Enterobacteriaceae*. *Int. J. Syst. Bacteriol.* 29, 352 - 360.
55. Vaidya S. A., Citron D. M., Fine M. B., Murakami G. and Goldstein E. J. (2006). Pelvic abscess due to *Ochrobactrum intermedium* [corrected] in an immunocompetent host. (2007) case report and review of the literature. *J. Clin. Microbiol.*; 44(3):1184-6. Review. Erratum in: *J. Clin. Microbiol.* 45(5):1672.
56. Van der Hoeven R., Betrabet G. and Forst S. (2008) Characterization of the gut bacterial community in Manduca sexta and effect of antibiotics on bacterial diversity and nematode reproduction. *FEMS Microbiol. Lett.*; 286(2):249-56.
57. Velasco, J., Romero, C., Lopez-Goni, I., Leiva, J., Diaz, R. and Moriyon, I. (1998). Evaluation of the relatedness of *Brucella* spp. and *Ochrobactrum anthropi* and description of *Ochrobactrum intermedium* sp. nov., a new species with a closer relationship to *Brucella* spp. *Int. J. Syst. Bacteriol.* 48, 759 - 768.
58. Wang P. C., Mori T., Komori K., Sasatsu M., Toda K. and Ohtake H. (1989). Isolation and Characterization of an *Enterobacter cloacae* Strain That Reduces Hexavalent Chromium under Anaerobic Conditions. *Appl. Environ. Microbiol.* 55(7):1665-9.
59. Wheen L., Taylor S. and Godfrey K. (2002). Vertebral osteomylitis due to *Ochrobactrum anthropi*. *Intern. Med. J.*; 32(8):426-8.
60. Wi Y. M. and Peck K. R. (2010). Biliary sepsis caused by *Ochrobactrum anthropi*. *Jpn. J. Infect. Dis.*; 63(6):444-6.

11/4/2012

Does Erythropoietin Protect the Intestine against Ischemic/Reperfusion Injury in Rabbits?

Shahryar Hashemzadeh [1], Khosrow Hashemzadeh [2], Mohammad Hossein Somi [3], Ramin Nosrati [4], Monireh Halimi [5], Raheleh Aligholipour [1], Kamyar Ghabili [6]

[1.] Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[2.] Department of Cardiovascular Surgery, Shahid Madani Hospital, Tabriz University of Medical Sciences, Tabriz, Iran
[3.] Liver and Gastroenterology Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
[4.] Department of General Surgery, Tabriz University of Medical Sciences, Tabriz, Iran
[5.] Department of Pathology, Tabriz University of Medical Sciences, Tabriz, Iran
[6.] Physical Medicine and Rehabilitation Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
kghabili@gmail.com

Abstract: The protective effect of erythropoietin (EPO) on intestinal ischemic/reperfusion injury has been less studied. Therefore, the aim of the present study was to evaluate whether EPO has protective effects on the intestinal ischemic/reperfusion injury in rabbits. Thirty healthy male New Zealand white rabbits underwent clamping of the superior mesenteric artery for 60 minutes. Then, the animals were randomly divided into two groups: the control group (n=15) and the EPO-treated group (n=15). In the EPO-treated group, subcutaneous EPO (1000 IU/kg) was given 10 minutes before clamping, 30 minutes after clamping and immediately before declamping. Likewise, subcutaneous saline was injected as placebo in the control group. Blood sampling was performed before, at 2, 6 and 12 h after ischemic/reperfusion injury for biochemical analysis including interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) measurements. At 2, 6 and 12 hours after ischemic/reperfusion injury, a segment of distal part of the terminal ileum was surgically resected from the ischemic intestine for light microscopic study. At 2 and 6 hours after the ischemic/reperfusion injury, the mean plasma levels of IL-6 in the EPO-treated group were lower than those in the controls (P<0.05). However, the mean TNF-α levels were lower in the control group at 2 hours after the injury (P=0.01). In the EPO-treated group, the mean levels of IL-6 at 6 hours after the ischemic/reperfusion injury were significantly higher than those at 2 hours after the injury (P=0.02). Furthermore, the mean levels of IL-6 at 12 hours after the ischemic/reperfusion injury were significantly higher compared with those at 2 hours after the injury (P=0.04). Histopathological assessment revealed that Park's score at 12 hours after the ischemic/reperfusion injury was significantly lower in the EPO-treated group compared with the control group (P=0.001). In conclusion, EPO might exert a protective effect against ischemic/reperfusion injury in the rabbit model of intestinal ischemia.
[Hashemzadeh S, Hashemzadeh K, Somi MH, Nosrati R, Halimi M, Aligholipour R, Ghabili K. **Does Erythropoietin Protect the Intestine against Ischemic/Reperfusion Injury in Rabbits?** *Life Sci J* 2012;9(4):4791-4795] (ISSN:1097-8135). http://www.lifesciencesite.com. 720

Keywords: Ischemic/reperfusion injury; intestine; erythropoietin, interleukin-6; tumor necrosis factor-alpha

1. Introduction

Ischemic/reperfusion injury of the intestine is a serious disease which can be caused by many clinical conditions including acute mesenteric ischemia, intestinal obstruction, incarcerated hernia, small intestine transplantation, neonatal necrotizing enterocolitis, trauma, and shock (Yuan et al., 2011). It is believed that the injury caused by restoration of blood flow into the tissue is markedly more destructive than the ischemic damage itself (Guneli et al., 2007). This condition is extremely dangerous and is associated with a high risk of mortality and morbidity (Granger and Korthuis, 1995). Small intestinal mucosa is one of the tissues being most sensitive to ischemia-reperfusion injury. Although the exact mechanisms involved in the pathophysiology of ischemic/reperfusion injury have not been fully understood, apoptosis regulated by series of complicated intracellular and extracellular pathways seems to play an important role in the pathophysiology of intestinal ischemic/reperfusion (Brath eta l., 2011; Ortiz et al., 2003). In the process of apoptosis, cytokines belonging to the tumor necrosing factor (TNF) superfamily bind to and activate cell membrane death receptors (Ortiz et al., 1999).

Recent studies show that erythropoietin (EPO), a glycoprotein hormone essential for normal erythropoiesis, has a protective role in prevention of programmed cell death (Hashemzadeh et al., 2012). EPO binds to a surface receptor and begins a series of mechanisms leading to production and activation of certain anti-apoptotic proteins (Oda et al., 1998; Silva et al., 1999). The protective effect of EPO on apoptosis has been studied on different organs including intestine. Apart from its anti-apoptotic

features, protective effects of EPO on ischemic/reperfusion injury in tissues have been attributed to anti-oxidative, anti-inflammatory, and angiogenic characteristics of EPO (Hu et al., 2012; Wang et al., 2010; Fan et al., 2010; Manzoni et al., 2005). Although numerous investigations have targeted at the effects of EPO on ischemic/reperfusion injury in different tissues (Sharples et al., 2004; Solaroglu et al., 2004, 2003; Junk et al., 2002; Ergur et al., 2008; Karaca et al., 2009; Hashemzadeh et al., 2012), the efficacy of EPO on intestinal ischemic/reperfusion injury has been less studied. Therefore, the aim of the present study was to evaluate whether EPO has protective effects on the intestinal ischemic/reperfusion injury in rabbits.

2. Material and Methods

Thirty healthy male New Zealand white rabbits were included in the study in accordance with the NIH Guide for the Care and Use of Laboratory Animals and local institutional guidelines for humane use of animals in research. All rabbits were kept under standard laboratory conditions and examined by a veterinarian. The animals had free access to food and water and were kept in room temperature (Hashemzadeh et al., 2012; Somi et al., 2011; Jouyban et al., 2011; Ashrafi et al., 2013). The study protocol was approved by the ethic committee for experimental research at Tabriz University of Medical Sciences.

Feeding of the animals was stopped 24 hours prior to the induction of the intestinal ischemic/reperfusion injury and they received only water. Animals were kept under general anesthesia with intraperitoneal pentobarbital (50 mg/kg) and placed on temperature controlled surgery tables. The anesthetized animals were maintained on heating pads throughout the procedure. Following a midline laparatomy, superior mesenteric artery was occluded with an atraumatic microvessel clamp for 60 minutes. Then, the animals were randomly divided into two groups: the control group (n=15) and the EPO-treated group (n=15). In the EPO-treated group, subcutaneous EPO (1000 IU/kg) was given 10 minutes before clamping, 30 minutes after clamping and immediately before declamping. Likewise, subcutaneous saline was injected as placebo in the control group. All other conditions were similar in the two groups.

Blood sampling from the ear-vein catheter was performed before, at 2, 6 and 12 h after ischemic/reperfusion injury. Stored blood samples at -70°C were used for biochemical analysis including aspartate aminotransferase (AST), alanine aminotransferase (ALT), interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), blood urea nitrogen (BUN), and creatinine (Cr) measurements.

For histopathological evaluation, each studied group was subdivided into 3 groups of five. The animals in these groups underwent resection of a 3-cm segment of distal part of the terminal ileum from the ischemic intestine at 2, 6 and 12 hours after ischemic/reperfusion injury, respectively. All sections were stained with hematoxylin and eosin, and were assessed by a blinded pathologist using light microscopy. A histopathology score was recorded for each section based on Park's score: grade 0, no damage to villi; grade 1, occasional tips affected; grade 2, majority of tips affected; grade 3, majority of tips and some villi affected; grade 4, tips, mid and lower portions of the majority of villi affected (Mori et al., 2008).

Data were presented as mean ± standard deviation (SD). Statistical analysis was performed with statistical package for social sciences (SPSS) for windows version 16.0 using Independent Samples T Test. A P value <0.05 was considered statistically significant.

3. Results

Before the ischemic/reperfusion injury, the mean creatinine, LDH and AST values were higher in the EPO-treated group, while the control group had higher mean ALT values (P<0.05, Table 1). At 2 and 6 hours after the ischemic/reperfusion injury, the mean plasma levels of IL-6 in the EPO-treated group were lower than those in the controls (P<0.05, Tables 2 and 3). However, the mean TNF-α levels were lower in the control group at 2 hours after the injury (P=0.01, Table 2). In addition, the mean BUN and LDH levels were lower in the EPO-treated group at 2, 6, and 12 hours after the ischemic/reperfusion injury (P<0.05, Tables 2 and 3). In contrast, the mean creatinine levels were higher in the EPO-treated group at 2, 6, and 12 hours after the ischemic/reperfusion injury (P<0.05, Tables 2-4).

Table 1. The biochemical parameters before the ischemic/reperfusion injury (CRP, C-Reactive Protein; IL-6, Interleukin-6; TNF-α, Tumor Necrosis Factor-Alpha; BUN, Blood Urea Nitrogen; Cr, Creatinine; LDH, Lactate Dehydrogenase; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase)

	EPO-treated group	Control group	P value
CRP	0.06±0.25	0.00±0.02	0.37
IL-6	0.54±0.18	0.48±0.23	0.49
TNF-α	3.26±1.25	3.53±0.75	0.48
BUN	50.96±8.26	54.06±14.77	0.48
Cr	2.12±0.5	0.94±0.21	<0.001
LDH	198.02±73.75	127.78±21.02	0.001
AST	20.72±7.61	11.63±6.42	0.001
ALT	20.24±5.13	23.86±4.51	0.04

Table 2. The biochemical parameters 2 hours after the ischemic/reperfusion injury (CRP, C-Reactive Protein; IL-6, Interleukin-6; TNF-α, Tumor Necrosis Factor-Alpha; BUN, Blood Urea Nitrogen; Cr, Creatinine; LDH, Lactate Dehydrogenase; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase)

	EPO-treated group	Control group	*P* value
CRP	0	0.03±0.12	0.32
IL-6	0.4±0.12	0.79±0.53	0.01
TNF-α	6.78±5.89	2.52±0.82	0.01
BUN	60.74±6.99	81.27±10.37	<0.001
Cr	2.46±0.6	1.35±0.18	<0.001
LDH	901.25±187.85	1165.5±362.78	0.01
AST	33.17±7.13	80.04±107.67	0.1
ALT	30.71±7.13	36.04±11.14	0.13

In the EPO-treated group, the mean levels of IL-6 (*P*=0.02), LDH (*P*<0.001), AST (*P*=0.03), and ALT (*P*=0.02) at 6 hours after the ischemic/reperfusion injury were significantly higher than those at 2 hours after the ischemic/reperfusion injury. Furthermore, the mean levels of IL-6 (*P*=0.04), creatinine (*P*=0.003), BUN (*P*=0.04), LDH (*P*<0.001), AST (*P*<0.001), and ALT (*P*<0.001) at 12 hours after the ischemic/reperfusion injury were significantly higher compared with those at 2 hours after the ischemic/reperfusion injury. Between 6 and 12 hours after the ischemic/reperfusion injury, only the mean LDH (*P*=0.003), AST (*P*<0.001), and ALT (*P*<0.001) levels significantly increased in the EPO-treated group.

Table 3. The biochemical parameters 6 hours after the ischemic/reperfusion injury (NA, Not Available; CRP, C-Reactive Protein; IL-6, Interleukin-6; TNF-α, Tumor Necrosis Factor-Alpha; BUN, Blood Urea Nitrogen; Cr, Creatinine; LDH, Lactate Dehydrogenase; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase)

	EPO-treated group	Control group	*P* value
CRP	0	0	NA
IL-6	0.58±0.25	1.31±0.82	0.003
TNF-α	3.76±2.48	3.34±1.49	0.57
BUN	63.74±3.56	83.23±13.03	<0.001
Cr	2.74±0.57	1.61±0.18	<0.001
LDH	1222.1±150.8	1918.8±473.87	<0.001
AST	40.83±11.13	76.42±29.46	<0.001
ALT	36.86±7.32	38.7±11.13	0.59

Histopathological evaluation results of the present study are shown in Table 5. Histopathological assessment revealed that Park's score at 12 hours after the ischemic/reperfusion injury was significantly lower in the EPO-treated group compared with the control group (*P*=0.001, Table 5).

Table 4. The biochemical parameters 12 hours after the ischemic/reperfusion injury (CRP, C-Reactive Protein; IL-6, Interleukin-6; TNF-α, Tumor Necrosis Factor-Alpha; BUN, Blood Urea Nitrogen; Cr, Creatinine; LDH, Lactate Dehydrogenase; AST, Aspartate Aminotransferase; ALT, Alanine Aminotransferase)

	EPO-treated group	Control group	P value
CRP	0.06±0.25	0.04±0.15	0.73
IL-6	1±1.08	1.84±1.97	0.21
TNF-α	4.09±3.67	6.44±8.26	0.32
BUN	68.65±12.58	79.8±16.46	0.04
Cr	3.1±0.48	2.23±0.3	<0.001
LDH	1553.2±359.73	2021.1±613.61	0.01
AST	70.62±19.97	81.54±9.6	0.06
ALT	65.36±13	40.91±12.47	<0.001

Table 5. Histopathology scores in both studied groups

	EPO-treated group	Control group	P value
Score at 2 h	2±1.87	2.4±1.81	0.74
Score at 6 h	2±1.22	3±1.22	0.23
Score at 12 h	1.6±0.57	3.4±0.54	0.001

4. Discussions

The present study revealed that EPO might have protective effect on the intestinal ischemic/reperfusion injury probably through suppression of IL-6 production. This finding is parallel to that of the similar investigation by Mori and colleagues (2008). Mori et al. (2008) induced intestinal ischemic/reperfusion injury in rats followed by subcutaneous administration of EPO (1000 U/kg) and its nonhematopoietic derivative (asialoEPO). They concluded that EPO and asialoEPO had protective effects against intestinal ischemic/reperfusion injury through inhibiting apoptosis, and release of IL-6 and TNF-α (Mori et al., 2008). Likewise, other similar studies confirmed this protective effect of EPO on intestines. However, these studies differ from our and Mori and colleagues' investigations in terms EPO dosage and route of administration, and biochemical analyses. In a study on rats, Guneli and colleagues (2007) found that intraperitoneal administration of EPO (5000 U/kg) either at five minutes before the ischemia or at the onset of reperfusion resulted in protection against intestinal ischemic/reperfusion injury. They reached such a conclusion based on tissue indicators of decrease in oxidative stress and apoptosis, and improvement in tissue (jejunum) injury (Guneli et al., 2007). In another study by Sayan et al. (2009), intraperitoneal administration of recombinant human EPO (1000 or 3000 U/kg) 24 hours before the intestinal ischemic/reperfusion injury in rats led to significant physiological and histopathological improvements. The former was determined according

to the inhibition of oxidative stress and leukocyte infiltration following EPO injection (Sayan et al., 2009).

The results of the present study are consistent with those of the previous investigations highlighting the protective effect of EPO on ischemic/reperfusion injury in different tissues. Protection against the ischemic/reperfusion injury following EPO administration has been reported in retina (Junk et al., 2002), central nervous system (Solaroglu et al., 2003; Smith et al., 2011; Teng et al., 2012), myocardium (Calvillo et al., 2003), kidney (Yang et al., 2003), liver (Solaroglu et al., 2004), lung (Wu et al., 2006), testicle (Ergur et al., 2008), and ovary (Karaca et al., 2009). In contrast, our previous investigation on the limb ischemic/reperfusion injury failed to result in similar findings (Hashemzadeh et al., 2012). Based on the plasma IL-1β, IL-6, and TNF-α levels as well as the histopathological assessment, EPO had no protective effect on ischemic/reperfusion injury of the limbs in rabbits (Hashemzadeh et al., 2012).

In conclusion, EPO might exert a protective effect against ischemic/reperfusion injury in the rabbit model of intestinal ischemia. This protective effect might be attributed to the anti-inflammatory and antiapoptotic characteristics of EPO through IL-6 suppression.

Acknowledgements:

This study was financially supported by a joint grant from the Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Sciences, and the Liver and Gastroenterology Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

Corresponding Author:
Dr. Kamyar Ghabili
Physical Medicine and Rehabilitation Research Center,
Tabriz University of Medical Sciences, Tabriz, Iran
E-mail: kghabili@gmail.com

References

1. Yuan Y, Guo H, Zhang Y, Zhou D, Gan P, Liang DM, Chen JY. Protective effects of L-carnitine on intestinal ischemia/reperfusion injury in a rat model. J Clin Med Res. 2011;3:78-84.
2. Guneli E, Cavdar Z, Islekel H, Sarioglu S, Erbayraktar S, Kiray M, Sokmen S, Yilmaz O, Gokmen N. Erythropoietin protects the intestine against ischemia/ reperfusion injury in rats. Mol Med. 2007;13:509-517.
3. Granger DN, Korthuis RJ. Physiologic mechanisms of postischemic tissue injury. Annu Rev Physiol. 1995;57:311-332.
4. Brath E, Miko I, Nemeth N, Kovacs J, Peto K, Furka I. Effects of allopurinol and preconditioning on apoptosis due to ischemia-reperfusion on a double jejunum-segment canine model. Acta Cir Bras. 2011;26:186-193.
5. Ortiz A, Justo P, Sanz A, Lorz C, Egido J. Targeting apoptosis in acute tubular injury. Biochem Pharmacol. 2003;66:1589-1594.
6. Ortiz A, Lorz C, Egido J. New kids in the block: the role of FasL and Fas in kidney damage. J Nephrol. 1999;12:150-158.
7. Hashemzadeh S, Hashemzadeh K, Ranjbari A, Halimi M, Estakhri R, Aligholipour R, Ghabili K. Erythropoietin does not ameliorate limb schemia/reperfusion injury in rabbits. Afr J Pharm Pharmacol. 2012;6:1457-1461.
8. Oda A, Sawada K, Druker BJ, Ozaki K, Takano H, Koizumi K, Fukada Y, Handa M, Koike T, Ikeda Y. Erythropoietin induces tyrosine phosphorylation of Jak2, STAT5A, and STAT5B in primary cultured human erythroid precursors. Blood. 1998;92:443-451.
9. Silva M, Benito A, Sanz C, Prosper F, Ekhterae D, Nuñez G, Fernandez-Luna JL. Erythropoietin can induce the expression of bcl-x(L) through Stat5 in erythropoietin-dependent progenitor cell lines. J Biol Chem. 1999;274:22165-22169.
10. Hu L, Yang C, Zhao T, Xu M, Tang Q, Yang B, Rong R, Zhu T. Erythropoietin ameliorates renal ischemia and reperfusion injury via inhibiting tubulointerstitial inflammation. J Surg Res. 2012;176:260-266.
11. Wang Q, Pfister F, Dorn-Beineke A, vom Hagen F, Lin J, Feng Y, Hammes HP. Low-dose erythropoietin inhibits oxidative stress and early vascular changes in the experimental diabetic retina. Diabetologia. 2010;53:1227-1238.
12. Fan X, Kavelaars A, Heijnen CJ, Groenendaal F, van Bel F. Pharmacological neuroprotection after perinatal hypoxic-ischemic brain injury. Curr Neuropharmacol. 2010;8:324-334.
13. Manzoni P, Maestri A, Gomirato G, Takagi H, Watanabe D, Matsui S. Erythropoietin as a retinal angiogenic factor. N Engl J Med. 2005;353:2190-2191.
14. Sharples EJ, Patel N, Brown P, Stewart K, Mota-Philipe H, Sheaff M, Kieswich J, Allen D, Harwood S, Raftery M, Thiemermann C, Yaqoob MM. Erythropoietin protects the kidney against the injury and dysfunction caused by ischemia-reperfusion. J Am Soc Nephrol. 2004;15:2115-2124.

15. Solaroglu A, Dede FS, Okutan E, Bayrak A, Haberal A, Kilinc K. A single dose of erythropoietin attenuates lipid peroxidation in experimental liver ischemia-reperfusion injury in the rat fetus. J Matern Fetal Neonatal Med. 2004;16:231-234.
16. Solaroglu I, Solaroglu A, Kaptanoglu E, Dede S, Haberal A, Beskonakli E, Kilinc K. Erythropoietin prevents ischemia-reperfusion from inducing oxidative damage in fetal rat brain. Childs Nerv Syst. 2003;19:19-22.
17. Junk AK, Mammis A, Savitz SI, Singh M, Roth S, Malhotra S, Rosenbaum PS, Cerami A, Brines M, Rosenbaum DM. Erythropoietin administration protects retinal neurons from acute ischemia-reperfusion injury. Proc Natl Acad Sci U S A. 2002;99:10659-10664.
18. Ergur BU, Kiray M, Pekcetin C, Bagriyanik HA, Erbil G. Protective effect of erythropoietin pretreatment in testicular ischemia-reperfusion injury in rats. J Pediatr Surg. 2008;43:722-728.
19. Karaca M, Odabasoglu F, Kumtepe Y, Albayrak A, Cadirci E, Keles ON. Protective effects of erythropoietin on ischemia/reperfusion injury of rat ovary. Eur J Obstet Gynecol Reprod Biol. 2009;144:157-162.
20. Somi MH, Hajipour B, Abad GD, Hemmati MR, Ghabili K, Khodadadi A, Vatankhah AM. Protective role of lipoic acid on methotrexate induced intestinal damage in rabbit model. Indian J Gastroenterol. 2011;30:38-40.
21. Jouyban A, Shoja MM, Ardalan MR, Khoubnasabjafari M, Sadighi A, Tubbs RS, Agutter PS, Ghabili K. The effect of quince leaf decoction on renal injury induced by hypercholesterolemia in rabbits: a pilot study. J Med Plant Res. 2011;5:5291-5295.
22. Ashrafi H, Ghabili K, Alihemmati A, Jouyban A, Shoja MM, Aslanabadi S, Adl FH, Ghavimi H, Hajhosseini L: The effect of quince leaf (Cydonia oblonga Miller) decoction on testes in hypercholesterolemic rabbits: a pilot study. Afr J Tradit Complement Altern Med. 2013;10:277-282.
23. Mori S, Sawada T, Okada T, Kubota K. Erythropoietin and its derivative protect the intestine from severe ischemia/reperfusion injury in the rat. Surgery. 2008;143:556-565.
24. Sayan H, Ozacmak VH, Sen F, Cabuk M, Atik DY, Igdem AA, Ozacmak ID. Pharmacological preconditioning with erythropoietin reduces ischemia-reperfusion injury in the small intestine of rats. Life Sci. 2009;84:364-371.
25. Smith PD, Puskas F, Fullerton DA, Meng X, Cho D, Cleveland JC Jr, Weyant MJ, Reece TB. Attenuation of spinal cord ischemia and reperfusion injury by erythropoietin. J Thorac Cardiovasc Surg. 2011;141:256-260.
26. Teng Z, Lu J, Wei G, Liu Y, Zhang X. Protective effect of erythropoietin on spinal ischemia-reperfusion injury. Afr J Pharm Pharmacol. 2012;6:546-549.
27. Calvillo L, Latini R, Kajstura J, Leri A, Anversa P, Ghezzi P, Salio M, Cerami A, Brines M. Recombinant human erythropoietin protects the myocardium from ischemia-reperfusion injury and promotes beneficial remodeling. Proc Natl Acad Sci U S A. 2003;100:4802-4806.
28. Yang CW, Li C, Jung JY, Shin SJ, Choi BS, Lim SW, Sun BK, Kim YS, Kim J, Chang YS, Bang BK. Preconditioning with erythropoietin protects against subsequent ischemia-reperfusion injury in rat kidney. FASEB J. 2003;17:1754-1755.
29. Wu H, Ren B, Zhu J, Dong G, Xu B, Wang C, Zheng X, Jing H. Pretreatment with recombined human erythropoietin attenuates ischemia-reperfusion-induced lung injury in rats. Eur J Cardiothorac Surg. 2006;29:902-907.

11/14/2012

Antecedents of Competency Mapping in Industrial Sector

Dr. S.Srividhya [1], P. Viji [2]

[1.] Assistant Professor, School of Management Studies, Anna University,Regional Center Coimbatore, Jothipuram Post, Coimbatore – 641 047, Tamilnadu, India. Email: ssautcbe@gmail.com

[2.] Ph.D Research Scholar, School of Management Studies, Anna University,Regional Center Coimbatore, Jothipuram Post, Coimbatore – 641 047, Tamilnadu, India. Email: srpviji@yahoo.com

Abstract: This article comprehensively examines the competencies expected within the industrial sector with special respect tower section as this directly enhances the competence level of the workers and thereby reducing the prevailing gap between their expectation and perception. One hundred staff were selected who are operating within the floor level were surveyed through a structured form supported the previous study. three broad areas as well as the profile of the workers at the side of personal, institutional and environmental factors area unit thought of to investigate the antecedents of competence mapping in industrial sector and therefore the study disclosed the amount of expectation is larger than the amount of perception all told the higher than aforementioned factors, since the mean of expectation is larger than the mean perception. The analysis disclosed that the antecedents of competence mapping associated with all the three factors taken for the survey isn't up to the bench mark level of expectation with reference to the interview session with the workplace staff.
[S.Srividhya, P. Viji. Antecedents of Competency Mapping in Industrial Sector. *Life Sci J* 2012;9(4):4796-4800] (ISSN:1097-8135). http://www.lifesciencesite.com. 721

Keywords: Competencies, Competency mapping, Employee expectation, Employee perception

Introduction

The colossal changes in economical, social, technical, political and cultural state of affairs have resulted in globalization, where the global economy is in the midst of renovation. The overall alteration in the pattern has insisted the countries all over the world in to the toughest competitions. Due to the removal of trade barriers the competitions are at the increasing phase to compensate companies are looking for individuals who are highly competent. A competent workforce is the most important asset for any organization, therefore irrespective of the size of the company it has to focus both on HRM & HRD. Employees given greater diversity and liberty in their jobs feel both less stressed and more contented. The business today frames new policies to recruit and retain the highly competent employees as they are the imperative advantage of the organization and this competency is based on the education one gets during their course of study.

Competency is the need of the hour, all trade conference are based on competency sets. An individual to be successful is better to build a core competency that will see them through crisis. **Klemp (1980)** Competency is 'an underlying characteristic of a person which results in effective and/or superior performance on the job'.

Competency mapping can play a significant role in recruiting and retaining workforce as it gives a more precise scrutiny of the job needs, the candidate's potential and the variation linking the two, and the expansion and training needs to bridge the gaps. **Melissa Brewerton (2004)** describes policies could influence skills replaced with competencies.

Competency Mapping helps individual to:

- Recognize their competencies and evaluate with the market constraint.
- Practice well, based on the need before interviewing.
- Reveal self confidence.
- Secure the required inputs.
- Develop the competencies based on the organizational needs.

Diverse learning category could be viewed as there would be learners with different traits and characteristics. Integrative learning results in competencies that are directly applied as manifestation where the performance based on the learning is assessed.

Statement of the Problem

The antecedents of competency mapping in knitwear industrial sector may be a framework for all industrial related institutions to serve for better tomorrow. The technical advancements have compelled the industrial sector to be more and more competitive to serve the customer requirement. The Skill chart of an individual could be developed when the respective person knows his ability regarding one particular skill. On identification of the requirement

and availability, the individual and the organization will have some difference; and this gap has to be bridged for further improvement.

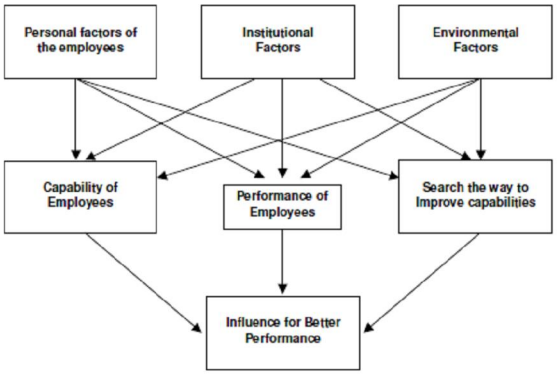
Conceptual Framework:

- ✓ Pradip N Khandwalla in his work "Competencies for senior manager roles" insists few examples played by senior level managers in turbulent business environment by revising the corporate growth and competitive strategies; this was the role Lou Gerstner played in the mid-1990s at IBM (Hartley, 1997).
- ✓ S Venkataramanan performed an equally audacious re-engineering job in the late 1970s on SPIC. SPIC was an Indian conglomerate, mainly into fertilizers and chemicals, whose promoters were the Chidambaram business group and the government of the state of Tamil Nadu. Venkataramanan, a bureaucrat, was brought in as the CEO to revive an ailing company (Ravindranath, 1985). Mr Krishnamurthy, CEO of a sick Steel Authority of India (Krishnamurthy, 1987) and Lawrence Bossidy of an ailing Allied Signal, US (Tichy and Charan, 1995) illustrate the kinds of leadership roles and competencies that can turn around sick organizations.
- ✓ Srivastava (2003), for instance, has sought to measure certain Indian business transformational leaders on some 50 such competencies and attributes. All the above models implies for successful leaders in due course of experience, on the other hand when the basic practical needs are fulfilled during the time of education, the students gets an opportunity to serve still better with his added experience.

Related Reviews:

- ❖ It has been identified that technological change as an important determinant of employment growth (Blanchflower *et al*., 1991) and of increased demand for more highly educated and skilled workers (Berman *et al*., 1997; Kiley, 1999; Machin *et al*., 1996).
- ❖ Surveys show a considerable increase in the average qualification levels of new recruits and an increase in job complexity (Green *et al*., 1997).
- ❖ Jobs were also found to require higher levels of skills than in the past within occupations, especially in professional and technological occupations (Osterman, 1995).
- ❖ Certainly, a diploma or license hanging on the office wall is no longer accepted by all as sufficient evidence of one's abilities to provide safe and effective care (Drotos, 2001).
- ❖ Substantial evidence proves that our educational systems have not kept pace with the dramatic changes (Hoge, 2002).
- ❖ Looking beyond the formal classroom to the realm of continuing education, research has shown that the prevalent teaching format, the didactic lecture, workshop, or conference, tends to neither change a provider's practice nor improve consumer outcomes (Davis & Taylor-Vaisey, 1997).
- ❖ Qualifications were to be grounded in an analysis of the important work behaviors and desired outcomes of the job (Equal Employment Opportunity Commission, 1978; Harvey, 1991; Shippmann et al., 2000).

Proposed Model



Objectives

1. To examine the required competencies of the employees' in knitwear sector in a globalized scenario.

2. To analyze the competency gap existing among the employees'.

Research Methodology

100 employees were selected who are working in the floor level were surveyed through a structured questionnaire were used for the collected works of data. T test was used to examine the major differences between variables and means of variables. For the reliability analysis Cronbach Alphas were used.

Cronbach Alpha coefficient is invented by Professor Cronbach, and this is an assessment of squared correlation among observed scores and true scores. The theory following is the observed score is equal to the true score plus the measurement error. A reliable test has to curtail the measurement error so that the error is not highly correlated with the true score, the relationship between true score and observed score should be strong, where the Cronbach Alpha examines this relationship, using SPSS software packages.

Questionnaire method was adopted to analyze the antecedents of competency mapping in the industrial sector, considering the employees' profile along with institutional & environmental factors, to know the impact on the capacity and performance. Some analysts suggest that necessary reading, writing and mathematics skills are no longer sufficient for workplace performance, but they are the opening point.

The competency mapping indicates the difference between the level of expectation & perception on the factors required for their competency development through their educational system. The competency mapping is measured with the help of personal (including the profile of the employees'), institutional and environmental factors. The relevant variables are drawn from the review of previous studies and they are discussed below

Table: 01 – Respondents Profile

Profile	Classification	Respondents	%
Gender	Male	52	52
	Female	48	48
Type of family	Nuclear	73	73
	Joint	27	27
Education	No Education	33	33
	High School	60	60
	Diploma / Degree	7	7
Nativity	Rural	40	40
	Semi-urban	32	32
	Urban	28	28
Family Income	<Rs.5000	22	22
	Rs.5000-10000	40	40
	Rs.10000-20000	17	17
	>Rs.20000	21	21
No of earners in the family	One	59	59
	Two	27	27
	Three and more	14	14

Out of 100 respondents 52 are male & 48 are female. The joint family set up is followed by 27% of the respondents' and the rest73% of the respondents' belongs to nuclear family. Around 40% belongs to rural area, and 40% belong to the income group of 5000 – 10,000, 59% are the only source of earning to the family.

Antecedents of competency mapping:

Personal Factor

The competency of the employees' is decided on the development of their personal factors. In the present study, the personal factors are measured with 5 variables namely **Media exposure, Sociability, Scientific orientation, Risk orientation and learning intensity**. The respondents are asked to rate the above set variables at five point scale on their level of expectation **(LOE)** and the level of perception **(LOP)** The mean score with mean deviation and standard error shows **(MD & SE)** the level of expectation and perception on personal factors related with their competency along with its statistical significance among these two. The results are shown in the Table: 02

Table: 02 - Personal factors

Statements	LOE	LOP	MD	SE	't'
Media exposure	4.23	4.27	0.04	0.100	(-.398
Sociability	4.10	3.92	0.18	0.108	1.672
Scientific orientation	4.27	3.86	0.41	0.106	3.851**
Risk orientation	4.17	4.12	0.05	0.129	0.388
Learning intensity	4.22	3.91	0.31	0.327	0.949

** Significant at 1% level

In the above said variables in personal factor, the level of expectation is greater than the level of perception since the mean of expectation is greater then the mean of perception. The mean differences are positive in nature. The higher mean difference is identified in the case of scientific orientation since its mean difference is 0.41 which is also significant at 5% level. In all other cases, there is no statistical significance between the level of expectation and perception on the variables in the personal capability factor. The above investigation reveal that the competency mapping related to personal factor is not up to the level of expectation of the employees.

Institutional Factors

Since the competency mapping among the employees depends upon the level of expectation and perception on the variables related to institutional factors among the employees. The mean score of the variable in environmental factor based on expectation & perception, its mean difference and its statistical significance have been computed and presented in table: 03. According to Adams Sixth Sigma, all successful commerce has a perform of assembling data and subjecting it to thorough gap analysis. Gap analysis is suitably exploited when appraising the performance within all facets of an organization.

GAP ANALYSIS – PAIRED 't' test

Table: 03 - Institutional Factors

Statements	LOE	LOP	MD	SE	't'
Reputation	4.137	3.126	1.011	0.130	7.771**
Infrastructure	3.726	2.716	1.011	0.148	6.839**
Financial motivation	3.789	3.137	0.653	0.127	5.148**
Career growth opportunities'	4.432	2.789	1.642	0.171	9.627**

**-Significant at 1% level.

Higher mean differences are noticed in case of organizations providing career growth opportunities'. Significant mean differences between level of expectation and perception are noticed in case of organization reputation, infrastructure, and fair & reasonable pay and higher career growth opportunities'. The analysis reveals that the level of expectation of institutional factors is higher than the perception on it. Hence the competency gap is in negative which reveals that the organizations are not enriching the level of competency expected by the employees.

Environmental Factors

Since the competency mapping among the employees depends upon the level of expectation and perception on the variables related to environmental factors among the employees. The mean score of the variable in environmental factor based on expectation & perception, its mean difference and its statistical significance have been computed and presented in Table: 04

Table: 04 - Environmental Factors

Statements	LOE	LOP	MD	SE	't'
Peer interactions	4.032	3.126	0.905	0.119	7.617**
Ergonomic factors	3.874	2.779	1.095	0.144	7.623**
Administrative environment	3.842	2.884	0.958	0.133	7.205**

**-Significant at 1% level

Higher mean differences are noticed for ergonomic factors. Significant mean differences between level of expectation and perception are noticed for peer interaction and location. The analysis reveals that the level of expectation of environmental factors is higher than the perception on it. Hence the competency gap is in negative which reveals that the organizations are not elevating the level of competency expected by the employees.

Capacity & Performance:

Since the competency mapping among the employees depends upon the level of expectation and perception on the variables related to capacity and performance factors among the employees. The mean score of the variable in capacity and performance based on expectation & perception, its mean difference and its statistical significance have been computed and presented in table:

Table: 05 - Capacity & Performance

Statements	LOE	LOP	MD	SE	't'
Knowledge	3.958	3.253	0.705	0.122	5.763**
Developing their technical skills	4.295	3.168	1.126	0.129	8.735**
Prioritize the issues	4.011	3.084	0.926	0.129	7.184**
Analytical thinking	3.947	2.989	0.958	0.129	7.445**
Problem Solving	4.063	3.221	0.842	0.148	5.704**
communication	3.895	2.958	0.937	0.139	6.758**
continuous learning	8.726	3.021	0.705	0.132	5.340**
Working ability	4.042	3.116	0.926	0.133	6.953**
social & ethical related issues	3.905	2.937	0.968	0.129	7.500**

**-Significant at 1% level.

Higher mean differences are noticed for interest in developing their technical interest. Significant mean differences between level of expectation and perception are noticed. The analysis reveals that the levels of expectation of the capacity of the employees' are higher than the performance perception on it. Hence the competency gap is in negative which reveals that the organizations are not enriching the level of performance to their maximum capacity.

Ways to improve performance

Since the competency mapping among the employees depends upon the level of expectation and perception on the variables related to the ways to improve the performance of the employees. The mean score of the variable in ways to improve the performance based on expectation & perception, its mean difference and its statistical significance have been computed and presented in table: 06

Table: 06 - Ways To Improve The Performance

Statements	LOE	LOP	MD	SE	't'
Focus	4.200	3.050	1.147	0.130	8.796**
Knowledge on latest tools & strategies	4.032	2.937	1.095	0.139	7.885**
policies & procedures	3.674	2.716	0.958	0.133	7.205**
professional development	4.010	2.726	1.284	0.138	9.326**

**-Significant at 1% level

Results & Discussions

Higher mean differences are noticed for concentration on overall development. Significant mean differences between level of expectation and perception are noticed. The analysis reveals that the level of expectation on the ways to improve performance is higher than the perception on it.

Hence the competency gap is in negative which reveals that the organizations are to take effective measures to improve the performance of the employees.

Research Implications

The findings of the present study regarding the antecedents of competency mapping in the industrial sector on the various factors like Institutional, educational, capacity & performance and ways to improve will enhance the competencies of the employees' to face the current situation and to reduce the existing gaps of competencies. Boyatzis (1982) and Fogg (1999) extend this definition to include both internal and external constraint, environment, and associations connected to the job or profession.

Motivations and perceptions of the work and one's self or talent also are viewed as powerful in expertly and successfully performing in a position. The results of the present study states that the employee with basic antecedes of competency mapping will be more competent and committed than the organization not possessing the basic eligible requirements.

Managerial Implications

The result of the present study was projected to support policy creators and decision makers in formulating policies and scheme that improve the individual employee well being leading to individual performance and organizational performance and environmental performance.

The present study identified 5 important areas that build for the positive improvement of the antecedents of competency mapping in the industrial sector, focusing on these will certainly minimize the gap existing among the employees'

Findings & Suggestions

Employee competencies decide the structure quality and also the entrepreneurial base of the state. In assessing the competencies of the staff enhancements are needed all told the three segments of non-public, Institutional and environmental factors are identified; the aim of this assessment is to let the staff and also the establishments grasp their needed competencies to match the performance to the expectation. Integration a holistic model for the commercial sectors might facilitate the staff to satisfy up to their expectation.

Author information:
Dr. S.Srividhya
Assistant Professor, School of Management Studies,
Anna University,Regional Center Coimbatore,
Jothipuram Post, Coimbatore – 641 047, Tamilnadu,
India. Email: ssautcbe@gmail.com

References

1. Afifi, A. A., & Elashoff, R. M. (1966). Missing observations in multivariate statistics. Part I. review of the literature. *Journal of the American Statistical Association, 61,* 595-604. Equal Employment Opportunity Commission, Civil Service Commission, Department of Labor, Department of Justice (1978). Uniform guidelines on employee selection procedures. Federal Register, 43(166), 38295–38309.
2. Nunnally, J. C. (1978). *Psychometric theory (2nd ed.)*. New York: McGraw-Hill.
3. Hartley, R (1979). "IBM: A Giant Falters, But then Rises,"in Harley, R, *Management Mistakes and Successes*, New York: John Wiley, 38-57.
4. Boyatzis, R. E. (1982). The competent manager: A model for effective performance New York: Wiley.
5. Ravindranath, S (1985). "SPIC Bounces Back", Business *India*, April 23-March 5, 110-120.
6. Krishnamurthy, V (1987). "SAIL Blazes a New Trail," *The Economic Times*, November 19, 5.
7. Blanchflower, D, Millward, N. and Oswald, A. (1991), "Unionism and Employment Behaviour", *Economic Journal,* Vol. 101, No. 407, pp. 815-834.
8. Osterman, P. (1995), "Skill, Training, and Work Organization in American Establishments"*Industrial Relations*, Vol. 34, No. 2.
9. Machin, S., Ryan,A. and Van Reena, J. (1996), Technology and Changes in Skill Structure, Evidence from an International Panel of Industries", Center for Economic Performance, Discussion Paper Series, London School of Economics and Political Science.
10. Green, F., Ashton, D., Burchell, B., Davies, B. and Felstead, A.(1997), "An Analysis of Changing Work Skills in Britain", Center of Economic Performance, Discussion Paper Series, London School of Economics and Political Science.
11. Fogg, C. D. (1999). Implementing your strategic plan: How to turn "intent" into effective action for sustainable change New York: American Management Association.
12. Kiley, M. (1999), "The Supply of Skilled Labour and Skilled-biased Technological Progress",*The Economic Journal*, No. 109, October, pp. 708-724.
13. Shippmann, J., Ash, R., Battista, M., Carr, L., Eyde, L., & Hesketh, B., et al. (2000). The practice of competency modeling. Personnel Psychology, 53(3), 703–740.
14. Srivastava, M (2003). *Transformational Leadership*, Delhi: Macmillan India.
15. Fulmer, R. M., & Conger, J. A. (2004). Identifying talent. Executive Excellence, 21(4), 11.
16. Lean Six Sigma Trainingwww.hbservices.in.

11/14/2012

Relation of creativity, emotional intelligence and social adjustment with elementary student's academic improvement in Iran

Somayyeh Hosseinpour [1] Mostafa Sheikhzade[2], Roqiyeh Vahdat[3]

[1]Department of Education, Urmia Branch, Islamic Azad University, Urmia, Iran
[2]Department of Education, Urmia Branch, Islamic Azad University, Urmia, Iran
[3]Department of Education, Urmia Branch, Islamic Azad University, Urmia, Iran

Abstract: Purpose of this article is to investigate the relation between creativity, emotional intelligence and social adjustment with fifth grade elementary students, education and training zone 1, in academic year 90-91.This survey is conducted with descriptive- correlation method on 312 fifth grade girl and boy elementary students that are selected with class sampling method.Variables of study are analyzed in social adjustment field through Shring emotional intelligence questionnaire, Abedi creativity questionnaire and California testing personality. Founded results indicate that there is no significant relation between emotional intelligence and academic improvement in fifth grade girl and boy elementary students (0.728) and (0.258). There is no significant relation between creativity and academic improvement in fifth grade boy elementary students (0.523). Also there is no significant relation between social adjustment and academic improvement in fifth grade girl and boy elementary students (0.363) and (0.920). According to significant level 0.003, it can be claimed that emotional intelligence is significantly more in boy students (101.31) than girl students (95.37) and this difference is statistically significance..
[Somayyeh Hosseinpour Mostafa Sheikhzade, Roqiyeh Vahdat. **Relation of creativity, emotional intelligence and social adjustment with elementary student's academic improvement in Iran**. *Life Sci J* 2012;9(4):4801-4810] (ISSN:1097-8135). http://www.lifesciencesite.com. 722

Keywords: creativity, emotional intelligence , social adjustment , elementary student's academic

1.Problem expression

Academic improvement as a training phenomenon is not affected with one factor. Even various factors such as scholastic aptitude, cognitive factors like (generic intelligence, scholastic efficacy, social adjustment, self-regulation strategies (Akinb, 2005), classroom structure, scholastic motivation) capability of learners, teachers education and learners motivation affect it (Mayee, 2001). Researches show that among various factors, training and individual factors with cognitive and social natures have the most effect on academic improvement (Kate and Kool, 1992, Lefransova, 1997, Seyf quotes, 1390). Golman (1998) defines emotional intelligence as containing capacities such as self-stimulation, resistance in facing with failures, momentums control and postpone of joys, setting your mood, sympathy and hope. He also defines emotional intelligence as capacity of recognizing your feelings and others feelings, self stimulation and correct management of your emotions in various relations with other people. Most of these specialists emphasis on this matter that there is a close relation between emotional intelligence variables and academic improvement in different age periods among girls and boys, in such a way that with increasing or decreasing of one variable, some changes occur in another variable.

Importance of this matter caused emotional intelligence to become as important as analytical and cognitive intelligence and be able to affect on individuals academic and career anticipation (Akbarzadeh, 1383). (Torrance, 1959, Kefayet, quotes) says in creativity definition: creativity is a process including sensitivity in problems, fixes and inconsistencies, this sensitivity occurs after a problem or problems and then a probe gets started to find solutions for solving that problems and projecting hypothesis for this purpose, after that the said hypothesis and therefrom solutions are examined and modified if necessary and needed changes are done and finally the results of this examination are published. Education and pedagogy specialists are well benefactor on this matter that non- dynamic education and training that just transfer of mental records is done in it would not be able to develop productive and creative individuals, ideally and according to existing global situation. Creativity and social adjustment are of those factors that can be appear in both facilitating and providing roles in academic performance so that, noticing to the individuals talents and providing a field to indicate these talents making such a sense in school that develops outdoor ambiguity, contrast, inconsistency, failure and lack of control causes creativity and innovation extension and as a result of students social

adjustment also their innovation effect would be increased and vice versa if fields for developing students creativity are not provided and their social adjustment and it's relative factors are not investigated and studied, students' academic performance would be decreased. So one of the most important ways of increasing students' academic performance level is to make creative and flexible structure and providing needed field to strengthen and growth of emotional intelligence and also helping to students for establishing healthy and appropriate and consistent relationship with their own social environment(Yarmohammadiyan,1380).

Some researches are done about relation of some variables of this survey in Iran. Sharifi(1376) in the field of relation of individual and social adjustment and academic performance indicates that as a student is socially and individually more adjusted his academic performance is higher. RAzaviyanshad(1384) concluded that there is a positive and significant relation between parents academic monitoring, parents valuing of science and education, parents academic expectations, social class, academic goal, family dimension and students academic self-concept with academic performance. Another research shows that there is a positive relation between problem solving metacognitive knowledge and ability to solve problem (Jahromi and Jahromi, 1389). Karimi (1379) showed that comparing girls and boys in creativity indicates significant difference between these two sexes. So knowing variables such as emotional intelligence, creativity and social adjustment as relative factors with student's academic improvement and determining each person's rate of portion in students' academic improvement explanation is as fundamental question of this research.

2.Research method

Regarding topic and its identity with respect to goal, it's method of research is practical and regarding the way of collecting data it is among descriptive surveys that will be done with correlation research method. Because we are intended to anticipate academic improvement by noticing to variables such as emotional intelligence, creativity and social adjustment in regression analysis form. Social, sample and sampling method under study statistical social is all of the fifth grade girl and boy elementary students in educational and training zone 1 that are educating in academic year 90-91. For computing sample volume of above society depending on variables quantitative features in this study (academic improvement) Cachran formula is used by considering all individuals of the society (n).

Table 1- society and computed sample

N	Social N	gender
159	1677	girl
153	1585	boy
312	3262	

2.1.Information collecting tools:

2.1.1. emotional intelligence test

For weighing emotional intelligence variable, Shring emotional intelligence questionnaire is used. This questionnaire is made in 1994. In this questionnaire some modifications are made and it is organized according to Iranian culture and also scoring method in this test is based on Likret five grade scale and choices always, most of times, sometimes, seldom, never respectively(1-2-3-4-5). Since emotional intelligence questionnaire is made by professional ones, it is verified by specialist professors and some of guidance counselors and advisers and it has a good content and form. Also for determining permanency of questionnaire it is done on a sample containing 30 individuals and cronbach's alpha coefficient is used that is computed equal to 85%.

2.1.2. social adjustment questionnaire:

In this survey California Testing Personality (CTP) is used in social adjustment field that is in yes-no question form and contains 90 questions that scoring system of test is as zero and one (no and yes). Currency and permanency of this questionnaire are evaluated in various researches. Masood nejad (1371) reports permanency coefficient of this test equal to 78% for social adjustment (masood nejad, 1371; Afshari quotes, 1375). Permanency of this questionnaire is performed on 30 persons in a primary study and through Cronbache's alpha computed equal to 0.64.

2.1.3. creativity questionnaire

Creativity questionnaire in Iran made for creativity measuring based on theory and definition of Gilford and Paul Torrance in current, innovation, flexibility and expansion 4 groups as a test containing 60 questions (Abedi, 1372). This tool contains 60 questions that 16 questions is in current part, 22 questions in innovation part, 11 questions in flexibility part and 11 questions in expansion part. Per question has 3 choices, score 1 belongs to first choice, score 2 to second choice and score 3 to third choice. These scores are collected in 4 groups and so 4 scores are obtained for (current, innovation, flexibility and expansion) parts that with summing 4

scores we can obtain full score of creativity for one person. Abedi (1372) performed this tool on 650 ones of Tehran's third grade guidance school and obtained permanency of this test through re-examination. Permanency coefficient for current part was 85%, for flexibility part 84%, creativity 82% and expansion 80%. Existing form of this test (60 questions) is used and examined by the professors of Spain Dosoto University and Cronbache's alpha internal consistency coefficient is used for permanency testing. This coefficient for testing of current part is equal to 75%, for innovation part 67% and for flexibility it is 61%.low permanency coefficient of this test is due to using internal consistency method, because replay of this in Dosto University is not possible. In order to measure permanency coefficient of creativity test (60 questions), an investigation is done on 30 second grade students of guidance school and after two weeks replay, the obtained coefficient is 82% in current part, 85% in innovation part, 88% in flexibility and 76% in expansion part. High permanency coefficient in this test indicates that using of replay can be a more appropriate way for computing permanency of test. Abedi (1382) conducted it on 650 ones of Tehran's students.
Torrance creativity test is also done beside this test on 200 persons of these students. Correlation coefficient between full score of Torrance test and full score of test obtained equal to 46%. Correlation coefficient between four scores of creativity analysis testing and academic scores, all were significant in 11 statistical level and was alternative between maximum 215% (between mathematic score and innovation part score) and minimum 54% (between mathematic score and flexibility part score).
Findings

3.Main question of research and Results

What is the share of emotional intelligence, creativity and social adjustment variables in explanation of student's academic improvement in fifth grade? For checking relation of predictor variables (emotional intelligence, creativity and social adjustment) with criterion variable (academic improvement) multivariable regression test is used (Table 2). Considering that significant level of related test is equal to 0/016, it can be claimed that the above test with 0/05 error in 0.95 confidence level is significant. This means that the choosed model in the form of (emotional intelligence, creativity and social adjustment) variables is significant so H1 concept is verified and considering the determination coefficient r2 that is ratio of explained changes through X variable to the whole changes is equal to 0/033, it can be stated that about 3 percent of students academic improvement changes are explained according to predictor variables (emotional intelligence, creativity, social adjustment, table 3).

Table 2- analysis of related variance to regression model of predictor variables (emotional intelligence, creativity and social adjustment) with criterion variable (academic improvement).

R		Distinction (R^2) coefficient	Adjusted distinction coefficient		Standard error	
0.181		0.033	-0.023		1.055	
Source of changes	Freedom degree	Sum of squares	Mean-square	F	Confidence level	Significant level
regression	308	343.39	1.115	3.47	0.95	0.016
remained	3	11.62	3.87		Test result H_0 Hypothesis acceptance	
total	311	355.01	------			

Table 3- parameter coefficient of first question related to predictive variables (emotional intelligence, creativity and social adjustment) with criterion variable (students' academic improvement)

Test result	significant level	calculated	line slope β	variable
	0.000	19.94	19.1	intercept
H_0 Hypothesis rejection	0.005	2.86	0.16	creativity
H_0 Hypothesis acceptance	0.758	-0.309	-0.018	adjustment
H_0 Hypothesis acceptance	0.292	-1.055	-0.059	Emotional intelligence

Table 3 shows that there is a significant relation between creativity variable and academic improvement (p<0/005), but there is no significant relation between emotional intelligence and social adjustment variables based on denotative coefficient (beta) and observed significant levels (p<0.292, p<0.758) and so computed regression equation will be equal to: (Academic improvement) Y= fix amount+ (0.163) creativity.

Subsidiary questions of research

1. Is there any relation between emotional intelligence with student's academic improvement in fifth grade?(table 4).

Significant level of r Pearson test was equal to 0.25 and this level is bigger than minimum significant level 0.05 and also by considering computed Pearson amount that is 0.092, this amount is smaller than r Pearson critical amount in 0.95 confidence level and 151, (0.139) freedom degree, so there is no significant relation between emotional intelligence and academic improvement in fifth grade boy elementary students. In another words the above hypothesis is not verified.

2 . Is there any relation between emotional intelligence with academic improvement in fifth grade girl elementary students?(table 5). R Pearson significant two dominant level is equal to 0.72 and this level is bigger than significant level 0.05 and according to computed Pearson amount that is 0.028, this amount is smaller than r Pearson critical amount in 0.95 confidence level and 157, (0.139) freedom degree, so there is no significant relation between emotional intelligence and academic improvement in fifth grade girl elementary students. In another words the above hypothesis is not verified.

3. Is there any relation between creativity and academic improvement in fifth grade boy elementary students? (table 6).

Table 4- correlation coefficient between emotional intelligence with academic improvement in fifth grade boy elementary students.

Numbers	Significant level	Correlation coefficient	Dependent variable	Independent variable
153	0.258	-0.092	Academic improvement	Emotional intelligence

Table 5-correlation coefficient between creativity with academic improvement in fifth grade boy elementary students

Numbers	Significant level	Correlation coefficient	Dependent variable	Independent variable
159	0.728	0.028	Academic improvement	Emotional intelligence

Table 6-correlation coefficient between emotional intelligence with academic improvement in fifth grade girl elementary students.

Numbers	Significant level	Correlation coefficient	Dependent variable	Independent variable
153	0.523	0.052	Academic improvement	creativity

As it is observed in above schedule r Pearson significant two dominant level is equal to 0.52 and this level is bigger than minimum 0.05 significant level and also according to computed Pearson amount that is 0.052 this amount is smaller than r Pearson critical amount in 0.95 confidence level and 151, (0.139) freedom degree, so there is no significant relation between creativity and academic improvement in fifth grade boy elementary students. In another words the above hypothesis is not verified

4. Is there any relation between creativity and academic improvement in fifth grade girl elementary students? (table 7).

r Pearson significant two dominant level is equal to 0.007 and this level is smaller than minimum 0.05 significant level and also according to computed Pearson amount that is 0.211 this amount is bigger than r Pearson critical amount in 0.95 confidence level and 157, (0.139) freedom degree, so there is significant relation between creativity and academic improvement in fifth grade girl elementary students. In another words the above hypothesis is verified.

5. Is there any relation between social adjustment and academic improvement in fifth grade boy elementary students?(table 8). r Pearson significant two dominant level is equal to 0.36 and this level is bigger than minimum 0.05 significant level and also according to computed Pearson amount that is 0.074 this amount is smaller than r Pearson critical amount in 0.95 confidence level and 151, (0.139) freedom degree, so there is no significant relation between social adjustment and academic improvement in fifth grade boy elementary students. In another words the above hypothesis is not verified.

6. Is there any relation between social adjustment and academic improvement in fifth grade girl elementary students?(table 9).

r Pearson significant two dominant level is equal to 0.92 and this level is bigger than minimum 0.05 significant level and also according to computed Pearson amount that is 0.008 this amount is smaller than r Pearson critical amount in 0.95 confidence level and 157, (0.139) freedom degree, so there is no significant relation between social adjustment and academic improvement in fifth grade girl

Table 7- correlation coefficient between creativity and academic improvement in fifth grade girl elementary students.

Numbers	Significant level	Correlation coefficient	Dependent variable	Independent variable
159	0.007	0,211	Academic improvement	creativity

Table 8-.coefficient between social adjustment and academic c improvement in fifth grade boy elementary students.

.

Numbers	Significant level	Correlation coefficient	Dependent variable	Independent variable
153	0.363	-0.074	Academic improvement	Social adjustment

Table 9- correlation coefficient between social adjustment and academic improvement in fifth grade girl elementary students

Numbers	Significant level	Correlation coefficient	Dependent variable	Independent variable
159	0.92	0.0008	Academic improvement	Social adjustment

Table 10- analysis of variance related to independent variables regression model (emotional intelligence, creativity and social adjustment) on dependent variable of this hypothesis (boy student's academic improvement).

R		Distinction coefficient (R^2)	Adjusted distinction coefficient		Standard error	
0.12		0.014	-0.005		1.2	
Source of changes	Freedom degree	Sum of squares	Mean-square	F	Confidence level	Significant level
regression	3	3.18	1.062		0.95	0.537
remained	149	217.5	1.46	0.722	Test result	
total	152	220.76	------		H_0 Hypothesis acceptance	

Table 11- parameter coefficient of seventh question related to independent variables (emotional intelligence, creativity and social adjustment) on boy student's academic improvement.

Test result	Significant level	canculated	line slope β	variable
	0.000	14.7	20.6	intercept
Hypothesis H_0 acceptance	0.628	0.486	0.04	creativity
Hypothesis acceptance H_0	0.464	-0.735	-0.061	adjustment
Hypothesis acceptance H_0	0.297	-1.04	-0.085	Emotional intelligence

elementary students. In another words the above hypothesis is rejected.

7. What is the share of emotional intelligence, creativity and social adjustment variables in explaining academic improvement in fifth grade boy elementary students? To investigate independent variables effect (emotional intelligence, creativity and social adjustment) on dependent variable of this research question (boy student's academic improvement) multivariable regression test is used (table 10).

As it is observed in Table 10, significant level of related test is equal to 0.537; this test with 0.05 error or 0.95 confidence level is not significant. So H1 hypothesis is rejected.

According to distinction coefficient R^2 that is explained changes ratio through X variable to the full changes, is 0.014, it can be claimed that about 1/4% changes in boy students academic improvement are explained through changes in independent variables(emotional intelligence, creativity and social adjustment). Table 11- parameter coefficient of seventh question related to independent variables (emotional intelligence, creativity and social adjustment) on boy student's academic improvement. So mathematical relation of emotional intelligence, creativity and social adjustment on fifth grade boy elementary students is as follows:

$Y= (0.040) X_1+ (-0.061) X2 + (-0.085) X_3$

So it is concluded that an increase in creativity causes 0.040 unit increase in boy student's academic improvement. Also with an increase in individual's adjustment, we see 0.061 decrease of standard deviation in boy student's academic improvement rate and with an increase in individual's emotional intelligence; we see 0.085 decrease of standard deviation in boy student's academic improvement rate.

8. What is the share of emotional intelligence, creativity and social adjustment variables in explaining academic improvement in fifth grade girl elementary students?

To investigate independent variables effect (emotional intelligence, creativity and social adjustment) on dependent variable of this research question (girl student's academic improvement) multivariable regression test is used (table12). Results of table 12 showed that, significant level of related test is equal to 0.054; it can be claimed that this test with 0.05 error or 0.95 confidence level is significant. So H1 hypothesis is verified. According to distinction coefficient R^2 that is explained changes ratio through X variable to the full changes, is 0.048, it can be stated that about 4/8% changes in girl students academic improvement are explained through changes in independent variables (emotional intelligence, creativity and social adjustment).

According to findings of schedule 13 there is a significant relation between creativity variable and academic improvement (p<0/006), but there is no significant relation between emotional intelligence and social adjustment variables based on denotative coefficients (beta) and observed significant levels

Table 12- analysis of variance related to independent variables regression model (emotional intelligence, creativity and social adjustment) on dependent variable of this hypothesis (girl student's academic improvement).

R		Distinction (R^2)coefficient	Adjusted distinction coefficient		Standard error	
0.219		0.048	0.03		0.87	
Source of changes	Freedom degree	Sum of squares	Mean-square	F	Confidence level	Significant level
regression	3	5.9	1.88	2.6	0.95	0.054
remained	155	118.6	0.76		Test result	
total	158	220.7			Hypothesis rejection H_0	

Table 13- parameter coefficient of seventh question related to independent variables (emotional intelligence, creativity and social adjustment) on girl student's academic improvement.

Test result	Significant level	computed t	line slope β	variable
	0.00	16.56	18.18	intercept
Hypothesis H_0 rejection	0.006	2.76	0.221	creativity
Hypothesis acceptance H_0	0.494	0.686	0.055	adjustment
Hypothesis acceptance H_0	0.720	0.359	0.028	Emotional intelligence

Table 14- T test results of two independent samples, about rate of emotional intelligence in girl and boy students.

Test result	Significant level	Freedom degree	t test	Standard deviation	mean	frequency	gender	variable
Acceptance	0.003	310	-2.98	16.66	95.37	159	girl	Emotional intelligence
				18.46	101.3	153	boy	

Table 15- t test results of two independent samples, about rate of creativity in girl and boy students.

Test result	Significant level	Freedom degree	t test	Standard deviation	mean	frequency	gender	variable
Acceptance	0.000	310	6.07	1.04	3.52	159	girl	creativity
				0.98	2.83	153	boy	

Table 16- t test results of two independent samples, about rate of social adjustment in girl and boy students.

Test result	Significant level	Freedom degree	t test	Standard deviation	mean	frequency	gender	variable
rejection	0.34	310	-0.94	2.27	28.13	159	girl	Social adjustment
				2.24	28.37	153	boy	

($p<0.292$, $p<0.758$) and so computed regression equation will be equal to:

(Academic improvement of girl students) Y= fix amount+ (0.221) creativity.

9. Is there any difference between emotional intelligence, creativity and social adjustment in fifth grade girl and boy elementary students?

Table 14- T test results of two independent samples, about rate of emotional intelligence in girl and boy students. Statistical analysis show that average of emotional intelligence among girl students (95.37) is different with average of emotional intelligence in boy students (101.31). Obtained findings indicates that t statistic of independent samples is equal to (t= -2.987) and yielded significant level (sig= 0.003) is satisfying, so with 0.95 confidence it can be claimed that emotional intelligence among boy students is significantly more than girl students. For comparing average of creativity rate in under study students in this survey, independent variable's t test is used (table 15). Statistical analysis show that average of creativity among girl students (3.52) is different with average of creativity in boy students (2.83). Obtained findings indicates that t statistic is equal to (6.076) and yielded significant level (sig= 0.000) is satisfying, so with 0.95 confidence it can be claimed that creativity among girl students is significantly more than boy students. Statistical analysis showed (table 16) that average of social adjustment among girl students (28.13) is equal to average of social adjustment in boy students (28.37). Obtained findings indicates that t statistic is equal to (t= 0.941) and yielded significant level (sig= 0.347) is not satisfying, because observed significant level is bigger than 0.05, so with 0.95 confidence it can be claimed that there is no difference between social adjustment among girl and boy students.

4.Discussion

Findings of survey are consistence with findings of a survey such as Halpern (2004); these findings are explained according to below probabilities. Categorizing coping styles into two emotion- focused and problem- focused styles don't possess the attribute of being preventive (Gaudreau & Blondin, 2002). Some researchers such as Anshel & Wiliams (2000) for analyzing coping styles used another scale that doesn't have this feature. With just having coping styles, it can't detect their differences, rather researchers such as Harren & Mitchell (2003) consider detector position role important. Finding of research are not aligned with findings of Rahnema and Abdolmaleki (1388) and also with research results of Parcer et al (2004), Elyas et al (2003), Beraket and Salvii (2004). Dichotomy of connection and disconnection of emotional intelligence with academic improvement that is observed in above results, can root in many factors such as cultural context of society, centralized and decentralized education system, basis variables(gender, academic grade, academic course…),situation of examination(method of research) and … .

Obtained results from relation of creativity with academic improvement is aligned with findings of Seyf (1386) and Abedi (1372) based upon disconnection between creativity and academic improvement but it isn't aligned with findings of Aknib (2005) and Kefayat (1373), Karimi (1379), Jahromi and Jahromi (1389) and Osare (1377) that indicated there is a positive and significant relation between creativity and academic improvement. Also there is a dichotomy about creativity in results that it also can root in many factors like cultural context of society, centralized and decentralized education system, basis variables (gender, academic grade, academic course …), situation of examination (method of research) and… .

Obtained results from relation of creativity with academic improvement is aligned with findings of Seyf (1386) and Abedi (1372) based upon disconnection between creativity and academic improvement but it isn't aligned with findings of Aknib (2005) and Kefayat (1373), Karimi (1379), Jahromi and Jahromi (1389) and Osare (1377) that indicated there is a positive and significant relation between creativity and academic improvement. Also there is a dichotomy about creativity in results that it also can root in many factors like cultural context of society, centralized and decentralized education system, basis variables (gender, academic grade, academic course …), situation of examination (method of research) and… .

Obtained results from relation of social adjustment with academic improvement is aligned with findings of Bronshtine et al (1993) Khodayarifard quotes (1385) and Behzadi (1365) and Heydari (1376) based upon disconnection between social adjustment and academic improvement but it isn't aligned with findings of Yarmohammadian et al (1381) and Berndt (1995), based upon connection of social adjustment with academic improvement. Also in this case there is a dichotomy in results that it also can root in many factors like cultural context of society, centralized and decentralized education system, basis variables (gender, academic grade, academic course …), situation of examination (method of research) and… .

References

1. Abedi, Jamal. (1372). Creativity and new ways in measuring it. Psychological researchs periodical. Second period. No 1 and 2.
2. Osare, Alireza. (1377). Learning and creativity. Peyvand monthly periodical. Parents and educators assembly no 233.
3. Akbarzadeh, Nasrin. (1383). Emotional intelligence: Salvii and others point of view. Tehran, Farabi publication.
4. Behzadi, Bahman. (1365). Analyzing academic situation of superme children of martyrs in guidance schools of Shriaz: martyer foundation of Islamic Revolution, office of research.
5. Dehshiri, Gholamreza (1385). Analyzing relation of emotional intelligence with student's academic improvement. News and research advices. 5 volume. No 18.
6. Golestan, Jahromi, Fatemeh and Golestan Jahromi, Masoomeh (1389). Analyzing relation of metacognition and creativity rate and student's academic improvement.
7. Heydari, Ahmad. (1376). Analyzing effective mental, social and educational factors on situation of martyr's students in guidance grade. Hamadan. Martyr foundation of Islamic Revolution. Office of research.
8. Karimi, Ali. (1379). Analyzing relation of creativity with anxiety, gender, academic improvement and social status of some of the third grade guidance school students. Master's thesis. Shiraz University.
9. Kefayet, Mohammad (1373). Analyzing realtion of methods and views of child development with creativity and relation of the last variable with first grade high school male student's sense and academic improvement in ahvaz.
10. Khodayarifard, Mohammad (1385). Analyzing effective factors on social adjustment of martyr's and normal university students. Tehran: psychology institution, Tehran University.
11. Nezami, Amir (1376).analysis of some effective social and psychological factors on high school

student's academic performance in Tabriz. Master's thesis, Shahid Bahonar University of Kerman, literature and human science faculty.
12. Rahnema, Akbar and Abdolmaleki, Jamal (1388). Analyzing relation of emotional intelligence and creativity with academic improvement in martyr university students. Modern training thoughts. Period 5. No 2.
13. Razaviyan Shad, Morteza (1384). Relation of emotional intelligence with social adjustment and academic improvement in third grade guidance school students in Tabriz. Academic year 83- 84. Master's thesis, Tabriz University, psychology and education sciences faculty.
14. Seyf, Ali Akbar. (1390). Developing psychology (education and learning psychology). Tehran: Agah publication.
15. Sharifi, A. (1376). Analyzing relation of satisfying emotional needs with individual-social adjustment and academic performance of third grade boy guidance students, Ahvaz township. Master's thesis, training psychology, psychology and education sciences faculty, Shahid Chamran university of Ahvaz.
16. Shahraray, Mehrnaz. (1373). In necessity of dynamic and creative training and education. Journal of humanities and social sciences. Shiraz university. Tenth period, no 1.
17. Yarmohammadian et al. (1380). Analyzing relation of mutual friendships, peer acceptance, self concept and social adjustment with girl student's academic improvement in third grade of guidance school, Esfehan.

11/2/2012

Wedding Celebration Customs of West Azerbaijan and Urmia in Tradition

Shamsi Miri Ghaffarzadeh [1], Hassan Nazari [2], Ninous Saadat

[1.] Urmia University of Medical Sciences, Urmia, Iran PO box 5714783734, Urmia, Iran
[2.] Department of Management, Mahabad Branch, Islamic Azad University, Mahabad, Iran
solarraha@yahoo.com

Abstract: There is a tradition, for each person before birth and after death that has shaped his life and put him in touch with people in his community. Some of these traditions are carried on a constant basis like many holidays and others are transient and in a stage of life like wedding celebration. Prior to modern times, arranged marriage process was one of the most important functions of the family through the direct involvement of family members, relatives or relatives search or by introducing marriage query donors. Traditional marriage customs were seen prevalent in a society and family played first role in choosing a spouse for their child People in traditional marriage relationships were supported by relatives' network In this paper an attempt has been made to codify the original sources and books, some of the customs and rituals relating to marriage.

[Shamsi Ghaffarzadeh, Hassan Nazari, Ninous Saadat. **Wedding Celebration Customs of West Azerbaijan and Urmia in Tradition**. *Life Sci J* 2012;9(4):4811-4814] (ISSN:1097-8135). http://www.lifesciencesite.com. 723

Keywords: customs, traditions, wedding, weddings, Urmia

1. Introduction

Many of the customs, traditions and beliefs of the people is rooted in history .Substantial part of the desired component of people's beliefs and folk is that they represents a community's cultural history and a testament to the depth dependence of the beliefs. Most customs has valuable and interesting meanings. Each part of Iran has its own way about marriage. The purpose of this ritual is the ceremony that comes with marriage. Even in primitive societies, the marriage of a girl and boy has not been taken away from the community and without ceremony. Traditional marriage is a process that has many steps. the traditional marriage which has been customary in most places, includes the following steps: Dating, matchmaking, formal matchmaking permission from the girl's family, Bale boran, buying wedding dowry, giving trousseau, henna night, preserving of bathroom, wedding ceremony, taking the bride to groom's house, the day after wedding, Madarzan salam and Pagosha.

2. Introducing

Many years ago, mother and sister of the one who wants to marry went to a traveling woman salesperson and gave her a mission to find a suitable girl. Badger women were to work as sales of pinned textile goods went into the house and analyze the girls patiently, and obtained the necessary information.

Then she sent it to the customer and made an acquaintance meeting discussions (1).

3. Woo

Many families preferred to go unannounced to woo to get more detailed information about the bride's family. In a surprising woo several females of groom relatives went to the house of the girl with candy filled bags or handkerchief ,then one of the women was knocking on the door and said: They show us a girl here. If the girl's parents were willing to accept woo, would offer the guests in. The surprising woos entertainment welcome had not done. If for any reason girl's family unwilling to accept unannounced woo, they told the negative answer then. In past, marriage of the younger girl was not customary until older sister was home and the other girls in the house were held off due to not to be chosen instead of the bride. In old surprising woos a woman of groom's family had been asked for water or tea, and expressed her and family desire to see the bride and then bride arrived with a tray of tea or bowl of water and was measured by the audience First, the appearance was analyzed that didn't contain any make up. Then determine how to entertain, decorum, shame and whether she is nice or not, whether she knows the household? Dose she follow in the older and then the younger? Then excuse for goodbye love kiss to determine whether her mouth or armpit smells bad. Also check her hair to not to be bald. The brief meeting ended with the pleasantries and goodbye and the two families messages were exchanged through the matchmaker (2). If the girl's parents agreed to a marriage proposal, commands for jam to be eaten as a sign of satisfaction. A few days later, the women would gather at the boy's home and were set marriage

contracts. In the mid-19th century, bride's father in affluent families asked for the sum of thirty Tomans from the groom for his daughter. If the preliminary talks would end up, wooer presented gifts. Here the bride and groom didn't see each other and when groom came to see her, never asked her about her acceptance and she would never dare to say no or to say: "I want to see a man whom going to live with me", if she dare to say, ignorant women surrounding her and began screaming: it's very bad for a girl to say that, girl's parents will marry their doughtier to whom they want". Sometimes the boy was able to see her face furtively from the roof of a neighbor. No boy was allowed to see his future wife face without mask. After the engagement the tip of girl's hair was cut and face was made up. From that day until the wedding boy should send holyday gifts holiday to girl like sheep on Eid. Sometimes a 14 year old boy was nominated to a 9 year old girl and sometimes as they was born and children stayed at their parents home until they reach the age of reason. In past, if a boy finishes his military service and then saw happy accepted the girl who was nominated for him and adults were responsible for it (3).

3.1 Formal permission of girls family for woo

If girl's family agrees woo, his father and several elders of the family went girl home and eat candy. The next day, mother, aunt, uncle or other close of groom went for Baleboron and were discussed on dowry, Shirbaha, wedding day and other issues. After several days, the groom's family sent gifts to the bride's house such as gold ring, with a bowl of candy, sugar some clothes for her father, mother and the bride's wedding clothes like: a chador, shirt and skirt, a pair of shoes, two pairs of socks and underwear. Some of the fruits and sweets were also count as a major part of these gifts (2).

3.2 Bale boron

Bale boron was and is an important part of wedding ceremonies traditional in Iran. This conference was held by the girl family who was married for first time to discuss about certain material conditions and boy's family's non cash and cash obligations of marriage. Bale boron is held in the context of economic so it had fundamental importance for marriage and two families' relationship bale boron was held after wooing and before attending the wedding at auspicious time and day at the girl's father or her older home. The parliamentary group of male and female elderly relatives of the two families coming together, and discuss about the amount of dowry, the marriage of toys, clothes and bridal jewelry, wedding and preliminary meetings expenses and number of guests and promises to be laid. If both parents agree, usually the written list of commitments made and signed by both parties seemed elders. Amount of dowry and wedding expenses and heavy style of their social and economic related to families, especially girl's family and clans that belong to (4).

3.3 Dowry

Traditionally, the bride in marriage time received money or property from the groom in kind of non-cash (gifts) (5). Dr M. Mohseni about dowry writes that dowry is the most important and most ancient tradition of marriage in Iran which has no place before Islam, but is accepted after that (6).

3.4 Wedding purchase

After wooing and parties' agreement and bale boron, the parties agreed on definite time that would not create trouble for purchasing wedding supplies, purchased devices were the mirror and candlesticks; make up instruments, underwear, shoes and socks and wedding accessories. To purchase these vehicles one or two of the family of the bride and groom were chosen and the bride and groom did not participate in the wedding equipment purchase and representatives buy needs for their taste. Groom will take these purchases charge (7).

3.5 Trousseau transference

A few days before the wedding, and sometimes the day before the ceremony, trousseau transference was done. Many users along with some mule and timpanist bring the dowry groom's home to do this. Before reaching of the group, some went home earlier to clean groom house for new furniture. When the bride's dowry was placed in groom's house, brides' family members show the dowry to the groom and took the receipt which is called Siyahe. Giving a tip to the herbicides and timpanist was considered among the duties of the groom. Sometimes the dowry items were: pots, pans, scoop, rinse, pitcher hip, bed, pillow, backpack, curtains, Samovar, crystal, mirrors and the Quran is considered one of the most important dowry items (7).

3.6 Hanabandan

Hanabandan was carried out three or four days before ceremony, at night girls and young females were beginning to dance in association with musicians and audience. after getting tired, near the middle of the night, hands and feet are tied to Hana Close of the morning, Hanabndan ceremony was ended and a group with the bride's family went to the bathroom, which previously had been grazed. Bridal first makeup was done in that night too (7).

3.7 Grazed bath

A bath was grazed for several hours by the family of the groom, and the bride's invitees, after Hana bndan ceremony went there and washed their henna packaged hands, feet and head. Usually all women and girls who stayed in bride's home and had gone to the bathroom at night, had breakfast in the

home of the bride and came back their home near noon and went back to her home after changing clothes to continue wedding . All costs and expenses of grazed bathroom, Hnabndan, breakfast and wedding day snacks was passed by the groom's family (7).

3.8 Wedding

Bride's family usually performed the ceremony and to do this they were conducted a room , before the ceremony, a number of stylish women and blessed families had decorated the room of wedding and placed wedding toys with a certain finesse. Toys for wedding ceremonies usually included as: transportation and sweets, a loaf, a few tulips or Jar Golabdan, Khoncheh, harmal and water bowls. During the marriage, the bride sat in front of the mirror and facing the Qiblah and when the conclusion of the sermon was read Parties are considered necessary for bride to look her in the mirror to clear her future as the mirror. Also in reading the conclusion of the sermon, two lucky women and kept a white cloth, double-overhead-fated on bride's head and another lucky women began to grate the suger on her head (8). The custom was that the girl was silent when was asked about the marriage until it was questioned three times. Time between the weddings and the party didn't take more than two days and they believe that if this period be more and of the members of the families die then the time would be increased (8). After the wedding music was played for few hours and people were spent a few happy hours, then a few members of groom family came to bring the bride with a white horse and horseback the bride. When the convoy was reached to the groom's house, groom should welcome the bride and throw an apple to her. If the bride could get the apple in the air it was a sign that the bride would dominate the groom. Then an underage boy brought a shovel containing some bread and cheese and wore it around the bride's waist and was sent the bride with the bread and cheese to groom's home. On arrival of the bride to the groom's house, hazelnuts or walnut to put under bride's foot to eliminate any spell. After the arrival of the bride to the groom's house, the women came and families were started to happiness. They took money and confetti on the bride and groom's head and believed that if any single girl ate the confetti she would be married soon. The groom was present in the women party and it was believed that he was intimate to all women in that night (9).

3.9 Patakhti and Madarzan Slam

One of the parties that was carried out after the wedding ceremony was Patakhti which had been done the night and the first morning after the wedding. Generally close relatives of the bride and groom were invited and attended the ceremony and some musicians were also invited to perform for the guests and made them happy the groom was required to have a meeting with his mother in law and has to thank because of the purity of her daughter (9).

3.10 Pagosha

Forty days after the wedding ceremony a party was held at the home of the bride's mother and the bride, groom and close relatives were involved. Organizing the party was to make both families more familiar and to the bride to come home again after a time (9). Marriages of Christians in rural extended families began in early fall because in this season farming ended and harvesting did. On the other hand, as the Christian prevented any celebration forty days before Noruz, the this period eating meat was not possible. Therefore they didn't celebrate weddings from a day after 18 February celebration hat was like Chaharshanbe Sori , to the fall of the following fall Wedding was lasted about 7 days later and was trying to held weddings on Fridays, Saturdays, Sundays. The customs of the time, if the wedding took place on a sunny day, it was omen and knew .rain an ominous on wedding day the groom's family usually female relatives went to the other house and invited them to the wedding and to knew the number of invitees gave a spoon to each family. However, there may also be invited collectively. However, musicians on the roof were invited all the villagers to the wedding. In addition, the groom would invite his single friends, and among them was a choice that was responsible for managing the work of the groom at the wedding "Coating groom's dress" ceremony was a ceremony in which the groom dresses would consecration by the priest .Then the groom was dressed by young men Usually the groom would have to wear his crown, a silk hat with a black and green, red and white cross which was engulfed in it.

At this time, any alley leading to the house of the bride, was closed by the bride's brothers and cousins and they had to open up, ask for their gift Sometimes the bride's family had brought a strong young man of their relative to wrestle with a young of the groom family that the story usually ended with the groom's representative admitted defeat On the other hand, the parents and relatives of the groom went to the bride's house. The bride's family kissed the groom and had served him with a piece of sugar or an apple to sweet his mouth. Wedding blessing was prepared and carried by a dancing woman. The bride's family' were welcomed the guests in yard. After that, the elder men and women came together in another room. The bride's family was prepared the means by which men were told "What is" questions and parables and hard puzzles were designed to be respond by the groom's young family members and to win the game. The stepfather and brother of the bride invited to come and her brother tied her girdle. In fact, like others they wanted

a boy to be their first child. Therefore, the bride's brother tied the symbolic number seven belts and said: “seven boys like me and one like you”. Therefore, the stepfather's wife plug a crown on bride’s head with seven pins which was symbolic function of the seven pins keep the bride from the evil eye and passion. After the ceremony, all started to cry with sad songs because the bride left the house of his father. Two new brides of the family came and began to dance with music. Priests and brothers of the bride took the bride's and groom hands and when the bride and groom face each other, pastor read sermon, though more formal wedding sermon was read in church. On this day, four singles of the bride family stole 4 object containing jar lid, ladle, spoon and Gata which each of these objects had a nice symbol, jar lid symbol of the low-letter and confidence, ladle was the symbol of magnanimity and homemaker wife, spoon represented symbol of parents home and Gata (Armenian bread) as a symbol of blessing and long live that brought by bride to the groom house. At this time, the bride's family and the other guests didn’t go to a church and remained there to break a cruse after the bride left to not to return to her father's home again, in other words, do not face the sadness of separation. Following the ceremony, the bride were carefully get on horseback and brought to the church and in church, was guided to the altar as the place where the marriage was that along with her groom. Priests close their heads together and tied with the green and red silk rope. Then kept the cross on their head and bringing out the knives and locks because passionate eyes could not be worked more. After church, the bride and groom were home In front of the groom's house, young and old, dance to show their duty to join the bride happiness in new home.

Certain foods such as butter and honey would give to the bride that she ate by her finger and then pass a cross on the door to have joy, goodness and happiness in the home The bride must entered groom’s home with her right foot and usually put a Lavash (kind of bread) on her shoulder to come home blessed .Here the bride and groom broke a ceramic pot to Finally, the bride and mother of the groom gifts where they could not hide out inclined On the wedding night, the bride and groom to stay awake because they believed that on this night, the beauty of the bride and groom to the bride and groom figures moved and violence are transmitted. So to prevent this tragedy, remained awake to welcome the sun and in the morning they both went. At the "Tajbrdary" was done the next day, the chaplain, father, and close relatives were invited to come and pastor silk canopy over the groom was. Thus the bride and groom after the couple were known in the family (10).

4. History of wedding traditions, to be prehistoric

This practice, common in many cultures of the world. The wedding ring exchange, exchange dowry and Shyrbha, gifts, flowers, dinner out on the wedding night, music, reading, praying and reading scriptures like Quran cultures of different nations in different forms and with different details are (11). In sum, the traditional marriage the family was satisfied with his choice to marry a guy with a girl, the boy was not allowed to marry And otherwise rejected families and marriages wife Kin selection as the traditional style, was very popular (12).

Corresponding Author:
Shamsi Miri Ghaffarzadeh
Urmia University of Medical Sciences, Urmia, Iran PO box 5714783734, Urmia, Iran.
E-mail: solarraha@yahoo.com

References

1. Massé H. Croyances et coutumes persanes: suivies de contes et chansons populaires. Librairie orientale et américaine; 1938.
2. Farhangi R. Cekbration of marriage. 1st ed.Tehran: Kelk-e-Azadegan; 2004. (Persian)
3. Marriage and matrimony [Internet]. 2012 [cited 2012 Nov 4]. Available from: http://forum.hammihan.com/ (Persian)
4. Beytoote. Bale boran [Internet]. 2012 [cited 2012 Oct 21]. Available from: http://www.beytoote.com/wedlock/danestani/outside-the-parliament-yes.html
5. Babakhani Z. The Dowry (mahr). Tehran: Ramin; 1998. (Persian)
6. Mohseni M. A survey on socico - culturalAttitudes in Iran. Tehran: golban; 2000. (Persian)
7. Literary Society Shafighi. A Traditional Urmia Marriage Ceremony [Internet]. 2007 [cited 2012 Nov 4].Availablefrom:http://www.shafighi.com/forum/showthread.php?t=4860 (Persian)
8. Mostofi A. Wedding tradition in Azerbaijan. Tehran: dibache; 2008. (Persian)
9. Goode. World Revolution and Family Patterns. Revised ed. Free Press; 1970.
10. Iranian Sociological Association. Mate selection in contemporary Iran and weddings with Armenians [Internet]. 2009 [cited 2012 Nov 4]. Available from: http://isa.org.ir/node/1213 (Persian)
11. borujerdtimes. Certain rituals of marriage between Iranian ethnic groups. 2012 [cited 2012 Nov 4]; Available from: http://www.borujerdtimes.ir/frhng-v-hnr/brrsy-brkhy-ayyn-hay-azdvaj-byn-aghva ayrany.aspx?Itemid=338 (Persian)
12. Sharifi A. dialectic accent Mokri. Tehran: Aubi; 1968. (Persian)

12/2/2012

Theoretical investigation of argon plasma formation induced by laser radiation

Kholoud A. Hamam , Haifaa M. AL-Ghamdi and Yosr E E-D Gamal[+]

Physics Department, Faculty of Science , King Abdul Aziza University , Jeddah , Saudi Arabia.
[+]National Institute of Laser Enhanced Science, Cairo University, Giza, Egypt
haifaa65@hotmail.com

Abstract: A previously developed electron cascade model is modified and applied to investigate the breakdown threshold for plasma formation and propagation in the focal volume . The study is devoted to investigate the measurement that carried out on the breakdown of argon over a pressure range 0.013-1.00 atm (10 -760 torr) induced by 532 nm of Nd:YAG laser with pulse length 8 ns and maximum energy 500 mJ. The model solves numerically the time dependent Boltzmann equation and set of rate equations that describe the change of the excited states population .The result showed good agreement between the calculated threshold intensities/ or laser input energy and the measured ones over the tested pressure range, this in turn validate the applied numerical model. More over the calculation of the EEDF and its parameters showed the correlation between gas pressure and physical processes responsible for the gas breakdown and plasma formation . Taken into consideration the spatial and temporal variation of the laser intensity in the focal volume it was possible also to present in this work the study of the effect of laser input energy on plasma propagation along the axial distance of the spatially varying focal volume. The result of this study illustrated the increase rate of plasma propagation by increasing the input energy, where it is found that at input energy equals three and half time its threshold energy value, the plasma propagates to cover the whole Rayleigh range in the backward direction.
[Haifaa M. AL-Ghamdi, Kholoud A. Hamam and Yosr E E-D Gamal. **Theoretical investigation of argon plasma formation induced by laser radiation.** *Life Sci J* 2012;9(4):4815-4822] (ISSN:1097-8135). http://www.lifesciencesite.com. 724

Keywords: argon, plasma pressure, plasma production by laser, electric breakdown, optical focusing, plasma diagnostics, laser –produced plasma, argon breakdown .

1. Introduction

The phenomenon of laser induced breakdown and plasma generation in gases have been studied extensively both experimentally and theoretically during the last five decades. Recently ,this phenomenon found a great importance for various applications, which include micro industries in electronics , environmental application for the measurement of pollution, surface cleaning, besides its application in medicine and biology. These studies showed that such applications are mainly depend on the characteristics of the plasma formed in the breakdown region. One of the main features of the formed plasma is its propagation in the backward direction of the focal volume as the input laser energy exceeds the threshold energy for breakdown. Moreover as the gas pressure increases the rate of propagation increases and more absorption of the input energy occurs in the plasma causing less transmission in the forward direction. Therefore more interest is devoted to study the physical processes responsible for this propagation (Yamada *et al.,* 1985;Yamada *et al.,* 1994;Tsuda *et al.,* 1997;Mlejnek *et al.,* 1999 ;Bindhu *et al.,* 2003). In these studies it was found that these physical processes depend on the parameters of the laser source as well as the nature of the irradiated gas.

Accordingly , A theoretical study of the phenomenon of laser induced breakdown and plasma generation in argon under the experimental conditions given by Bindhu *et al.* (2003) is presented . In this experiment the breakdown is obtained using a focused high intensity laser beam of wavelength 532 nm , pulse duration 8 ns and maximum energy 500 mJ , to irradiate argon gas over a pressure range varies between 10-760 torr, which is equivalent to 0.013-1.0 atm .This gas has been chosen since it has examined experimentally by various authors see for example: Yamada *et al.,* 1985;Yamada *et al.,* 1994; Tsuda *et al.,* 1996; Mlejnek *et al.,* 1999 ;Bindhu *et al.,* 2003). Moreover ,it showed a minimum Ramsuer, in the relation between the momentum transfer collision cross-section and the electron energy. This minimum might has a noticeable effect on rate of energy gain by electrons from the laser field during the Inverse Bremsstrahlung absorption process which plays an important role in the breakdown of argon (Gamal *et al.,* 1986). The investigation is based on a modified electron cascade model (Gamal and Azzouz 1987; Gamal *et al.,* 1999) which depends on the numerical solution of the time dependent Boltzmann equation simultaneously with a set of rate equations that describe the rate of change of the formed excited

states density. Calculation assumed first temporal variation of the laser intensity in a cylindrical focal volume with a Gaussian shape .This enables the study of the threshold intensity as a function of gas pressure. In addition a study of the temporal variation of the electron energy distribution function (EEDF) and its parameters during the laser pulse as a function of the gas pressure demonstrated the correlation between the physical processes responsible for the gas breakdown. The study takes also into account the effect of electron gain and loss processes at the different gas pressure values. To study the plasma propagation in the focal volume the model considered the temporal and spatial variation of the laser intensity along the axial and radial axes of the focal volume as a function of the laser input energy. This study clarifies the relation between the density of the formed plasma and its absorption rate of the laser input energy as well as its propagation rate in the focal volume.

2. Theoretical formulation

2.1 The model

A detailed description of the model is given in Evans and Gamal (1980) ; Gamal and Azzouz (1987) and Gamal *et al.* (1999). Here we summarize only the outlines of the model. The model is based on the assumption of the presence of at least one free electron in the focal volume at the onset of the laser pulse. This electron gains energy due to collision with neutral gas atoms in the presence of laser radiation through inverse Bremsstrahlung absorption. The energy gained from the laser field by electrons is given by, inelastic collision terms (1)

$$\frac{\partial n(\varepsilon,t)}{\partial t} = \frac{1}{3}\varepsilon_o \nu_m(\varepsilon)\frac{\partial n}{\partial \varepsilon} + \frac{2}{3}\varepsilon_o \varepsilon \nu_m(\varepsilon)\frac{\partial^2 n}{\partial \varepsilon^2} +$$

where ε0 =e2E2 /2mω2 is the average oscillatory energy of an electron in the laser field with electric field E and angular frequency ω, e and m are the electronic charge and mass, νm(ε) is the momentum transfer collision frequency and n(ε) is the electronic density at energy range ε , ε+dε.

The first term in the right-hand side corresponds to energy gain, the second term results in no net energy gain and is referred to as the energy diffusion along the axis.

Adopting the same assumption for the argon atom model given by Weyl and Rosen (1985), therefore, the argon atom is treated as a four-level atom such as: a ground state; an excited state 4S state at an energy loss 11.6 eV; an excited state 4P at an energy loss of 13.2 eV; and an ionized state at energy 15.75 eV.

Accordingly, the following inelastic collisional and radiative processes are taken into account: (1) Inverse Bremsstrahlung absorption; (2) electron impact ionization of ground state atoms with electrons having energies >15:8 eV; (3) electron impact excitations to the 4S and 4P states with electrons having energies > 11:6 eV and 13.2 eV, respectively; (4) photo-ionization of these formed excited states; and (5) collisional ionization of the excited states by electrons having energy > 4:65 eV for the 4S state and 2.85 eV for the 4 P state, respectively. Beside these excitation and ionization processes, loss processes such as electron diffusion out of the focal region and electron-ion recombination processes (three body recombination) are also included in this analysis.

2.2. Argon Cross sections and rate coefficients

The various relevant cross sections and rate coefficients of the argon gas considered in the present work were as follows.

The collision cross section of the momentum transfer for argon is taken from Gamal *et al.* (1986) where a curve fit expression was obtained using the experimental data giving by Hayatshi (1981) as

$_m$=10^{-15}/(1+144.6356 -2583.08 ^{2} + 35035 ^{3} -85386.8 ^{4}) for 0.0< < 0.3 eV (2)

and

$_m$=10^{-17}[11.467(e$^{-0.093}$) + 2:817(e$^{-0.093}$)^2 +0.693(e$^{-0.093}$)^3] cm^2

-Then the collision frequency n_m is related to the collision cross section s_m by the following relation

$_m$= N $_m$ (2 /m)$^{1/2}$ s^{-1} (4)

where N is the gas density per unit volume.

-For cross sections of excitation and ionization, we applied those which were considered by Weyl and Rosen (1985) as:

$_{Ex4S}$(=4.65x10^{-18} (cm^2 > 11:6 (5)

$_{Ex4P}$(=1.17x10^{-17} (cm^2 > 13.2 (6)

$_i$(=1.45x10^{-17} (cm^2 > 15.75 (7)

-Because of the lack of experimental data concerning the collisional ionization of excited states for argon, we have adopted the same assumption as made by Evans and Gamal (1984). Therefore, these cross sections are assumed to have the same analytical expressions as that of the ionization of the ground state atom with some adjustment for the numerical factor such as:

$_{i4S}$(=1.45x10^{-15} [cm^2≥(15.75-11.6) (8)

$_{i4P}$(=1.45x10^{-15} [cm^2 ≥(15.75-13.2) (9)

-Photo-ionization coefficients of the excited atoms are estimated from a formula given by Grey Morgan (1975) to be

$$C_q = \sigma^q/(q-1)\nu_L^{q-1}h^q \quad (10)$$

where σ^q is the atomic cross section (typically≈ 10^{-16} cm^2), ν_L is the laser frequency, h is Plank's constant and q is the number of photons absorbed by an atom to be ionized.

Under the experimental conditions of Rosen and Weyl (1987) (λ= 0.53μm) two photons are required to photo-ionize both the 4S state and the 4P state. Therefore, the estimated photo-ionization coefficient using this formula is given by

$C^2 = 1{:}27 \times 10^{-14}$ s−1 (W cm^{-2})$^{-2}$ (11)

which is in accordance with that given in Weyl and Rosen (1985). In this case the photo-ionization of the 4P state results in an electron with an energy ≈2 eV.

-The three-body recombination coefficient is taken from Weyl and Rosen (1985) as

$R_{3b}=5.6 \times 10^{-26} n/$ $^{9/2}$ cm^3 s^{-1} (12)

where n is the electron density.

-Finally, the electron diffusion coefficient is calculated using the same equation as that used by Rosen and Weyl (1987), namely

$$\nu_D \frac{2\varepsilon}{3m\nu_C}\frac{1}{\Lambda^2} \quad (13)$$

where Λ is the diffusion length and as in the present case when the cylindrical focal volume of radius b is considered, Λ is approximated by $\Lambda = b/2.405$ and is the average electron energy. Ambi-polar diffusion is ignored in this work as it is insignificant under the experimental conditions considered in this analysis (Weyl and Rosen, 1982).

The temporal variation of the laser intensity is taken as Gaussian shape and the focal volume is considered to be cylindrical with radius r and axial length z. .The breakdown criterion adopted in this work is the attainment of ionization 0 .1% of the atoms present in the focal volume. The spatial distribution of the laser intensity is considered to be varied with the length of the focal volume such as:

$W(z)= W_0 (1+z^2/z^2_R)^{1/2}$ (14)

Where z_R is the Rayleigh length.

Considering these cross sections and rate coefficients a computer program was undertaken to investigate experiments on the breakdown of argon by laser radiation.

3. Results and Discussion

Applying the considered model computations are conducted to obtain the threshold intensity for breakdown and plasma development as a function of the gas pressure. Comparison between these values and those experimentally measured by Bindhu *et al.* (2003) is shown in figure (1).Good agreement is obtained where both showed decrease of the threshold intensity/laser input energy as the gas pressure increases. This result confirms the validity of the model to investigate the experimental measurements considered in this study. In order to study the physical processes responsible for breakdown as a function of gas pressure figure (2) illustrates the EEDF calculated at the end of the pulse for different values of the gas pressure.

This figure showed that as the gas pressure increases the EEDF increases with its tail directed towards the energy range which is almost coincide with the ionization limits.

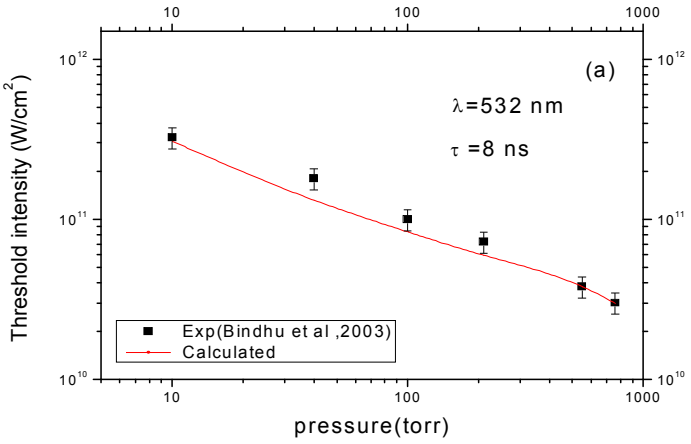


Fig.1: Comparison between the calculated and measured threshold intensities as a function of gas pressure

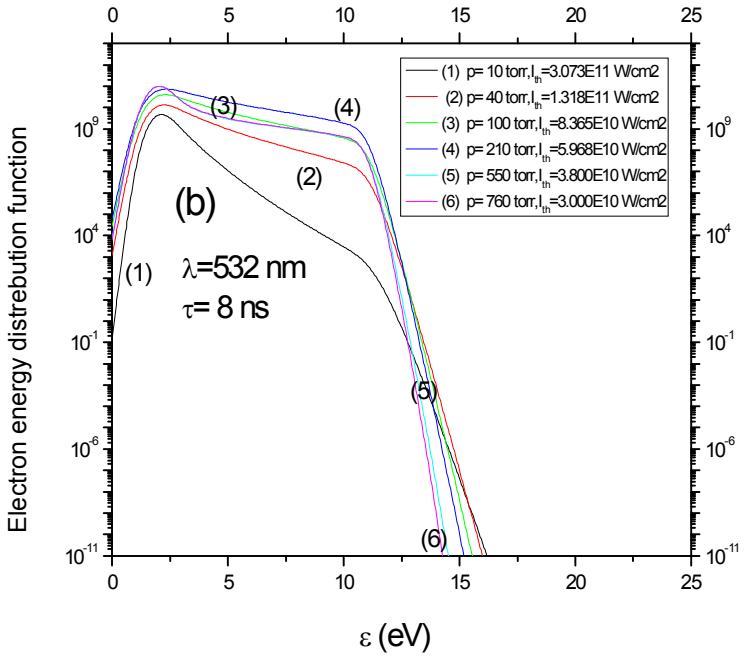


Fig.2. The EEDF calculated at the end of the laser pulse for different values of gas pressure.

To assure the correlation between the physical processes and gas pressure, figure (3) represents the time evolution of the electron density at the different pressure values. It is clear from this figure that at the low pressure value the electron density increases slowly during the early stages of the laser pulse .This is attributed to high competition rate between the generation of electros through photo-ionization process and its losses from the vocal volume by diffusion at this pressure range. As the gas pressure increases collision processes may contribute pronouncedly to the electron growth rate beside the photo-ionization process. This in turn illustrate the fast increase of the electron density near the peak of the laser pulse shown by curves(5) and(6) in this figure.

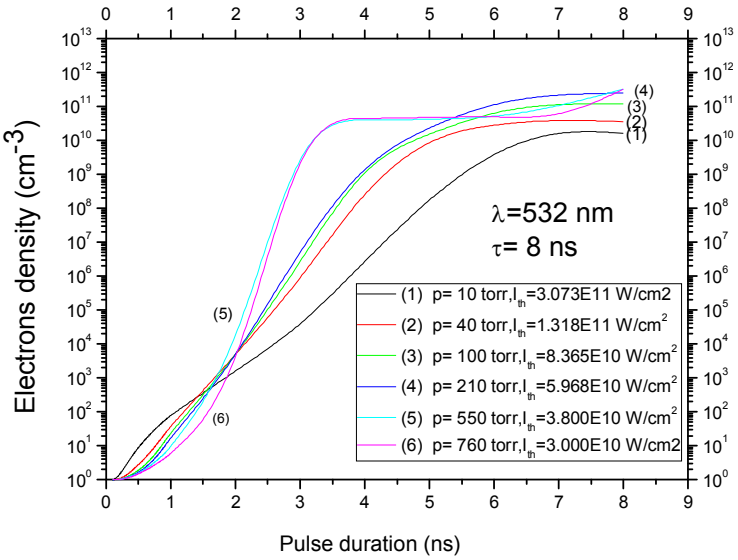


Fig.3. Time evolution of the electron density at the different values of the gas pressure.

In figure (4) the time variation of the electron mean energy is presented for the different pressure values. This figure illustrates the role played by the photo ionization process at the low pressure value , where the electron mean energy starts with high value(4 eV) then it decrease fast down to a value of 2 eV ,where it continue at this value up to the end of the pulse This confirms the fact that ionization at this pressure proceeds via photo ionization processes . At the intermediate pressures ,although the electron mean energy starts at the same value ,but it undergoes a fast decrease followed by a noticeable increase at the end of the pulse .At high pressures different behaviour is observed where the electron mean energy suffers from an increase around the peak of the pulse ,this clarifies the role of gain processes which could easily overcome the loss processes.

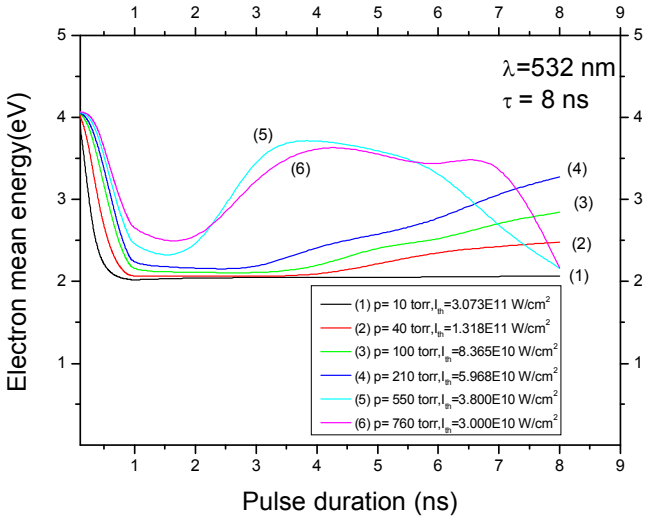


Fig.4: Electron mean energy plotted against time for the different values of the gas pressure.

To study the effect of the physical processes on the characteristics of the formed plasma ,figure (5) illustrates the contour image of the electron energy distribution zones that represent the formed plasma at gas pressure value 210 torr. This value is deliberately selected since it represent the most lengthy formed plasma among those calculated at different pressures.

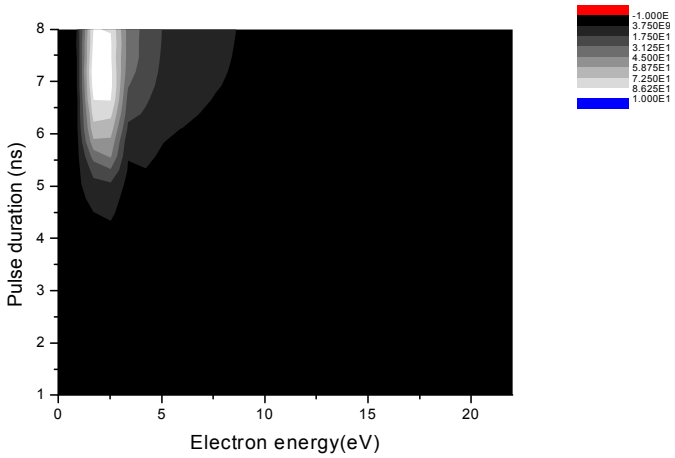


Fig.5 : Contour lines of the calculated temporal variation of the Electron energy distribution zones at pressure 210 torr.

To study the effect of gas pressure on the plasma propagation in the focal volume ,calculations are carried out to find out first a relation between the absorbed and scattered energy as well as the threshold intensity as a function of gas pressure covering a range 1.0 -100 atm (10 – 760 t0rr) at laser energy corresponds to the breakdown condition 230 mJ, (Yamada *et al.,*1994). This is shown in figure (6) ,where the absorbed energy reaches its maximum value at 760 torr. This indicates that the absorbed energy may exhaust in plasma expansion in the breakdown region under this experimental conditions.

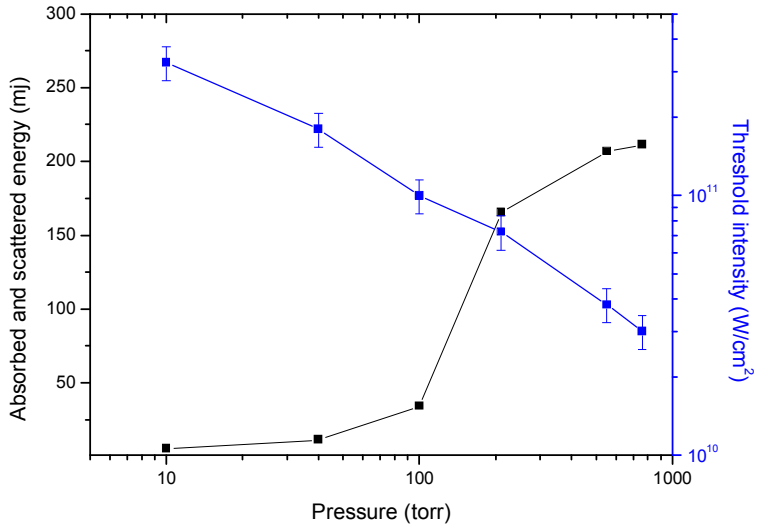


Fig. 6 Relation between the absorbed and scattered energy and the corresponding values of the threshold intensities plotted as a function of gas pressure.

Figure (7) shows the variation of the intensity as a function of the input energy at different axial points along the axial distance of the focal volume at laser input energies 12 mJ , 55 mJ and 155 mJ. It is noticed here that at the highest energy the plasma expands to a distance lies between 0 and z_R (The central point and the Rayleigh length). This means that as the input energy increases the plasma propagates more towards the laser beam.

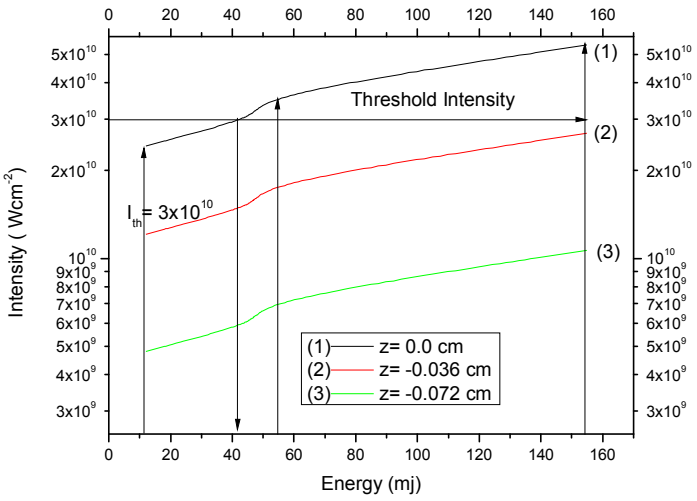


Fig.7. Variation of the intensity as a function of the laser input energy at different values along the axial distance.

To confirm this result a relation between the electron number along the axial distance at laser powers 7 MW and 20 MW is plotted in figures (8 ,9) to specify the actual axial distance at which breakdown occurs , this in turn identifies the length of the formed plasma .Increasing the laser power results in an increase of the plasma length despite the value of the gas pressure .

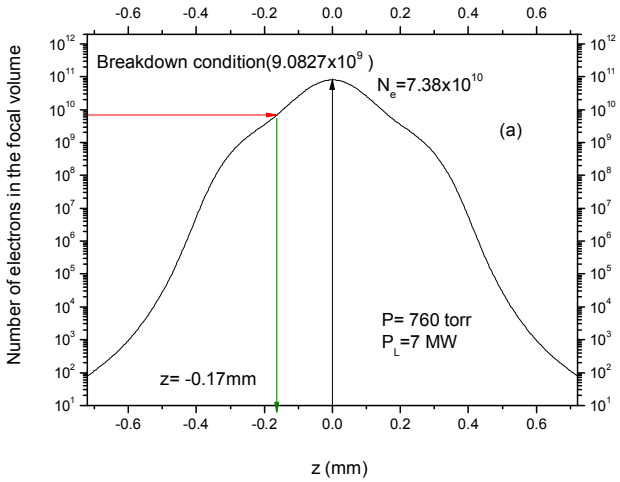


Fig.8 Variation of electron number as a function of both the axial and radial distances at input power 7 MW

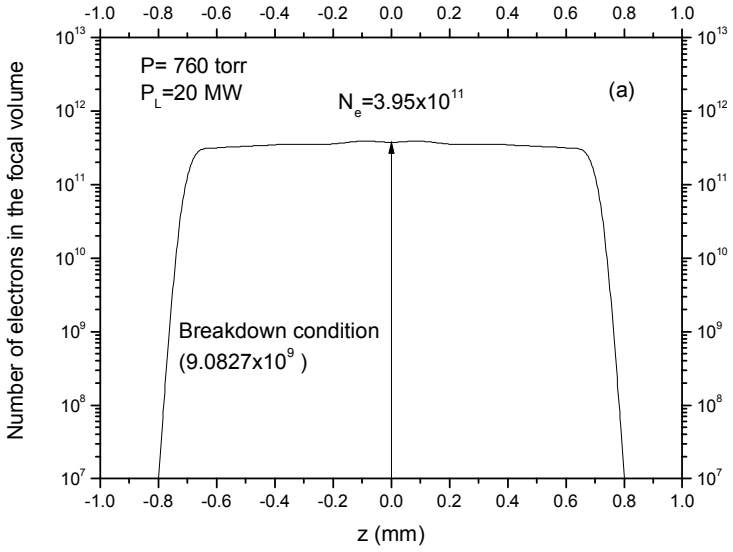


Fig. 9 The same as in figure 8 but at laser input energy 20 MW.

4. Conclusion

The electron cascade model presented in this work provided a reasonable interpretation on the effect of gas pressure on the physical processes responsible for the breakdown of argon over a pressure range 0.013-100 atm (10 – 760 torr) by the second harmonic of a Nd:YAG laser source with 8 ns pulse duration. This is confirmed from the good agreement which obtained between the calculated threshold intensity as a function of the gas pressure and the experimentally measured ones. The calculation of the EEDF and its parameters underlined the characteristics of the formed plasma in the breakdown region and its relation with the gas pressure. Electron diffusion acts to deplete the electron density at the low pressure regime. More over the study of the spatial and temporal variation of the laser intensity in the focal volume showed the exact correlation between laser input energy ,gas pressure and plasma expansion and propagation along the axial distance.

References

1. Bindhu C V, S S Harilal, M S Tillack, F Najmabadi and A C Gaeris, (2003): "Laser propagation and energy absorption by an argon spark" Journal of Applied Physics , 94,7402-7407.
2. Yamada, J. Takamichi, T. and Takayoshi, O. (1985) :"Physical Properties of laser-produced dense plasma in high pressure Argon gases." Japanese Journal of Appl. Phys. Vol. 24.856-861.
3. Yamada, J. Tsuda, N. Uchida, Y. Huruhashi, H. and Sahashi, T. (1994): "Study on Laser Induced Plasma Produced in Liquid". Trans. IEE Jpn. A 114.303.
4. Mlejnek, M. Wright,E.M. and Moloney, J. V. (1999): " Moving-focus versus self-wave guiding model for long-distance propagation of femtosecond pulses in air" IEEE J. Quantum Electron. ,35,1771-1776.
5. Evans, C. J. and Gamal, Y. EE-D . (1980) :"Laser-induced breakdown of helium". J. Phys. D: Appl. Phys., 13, 1447.
6. Hayatshi, M .(1981) :"Report on Atomic data" IPP/Univers. Of Nagoya Japan NOIPPJ-AM-19.
7. Weyl, G. and Rosen, D. (1985): "Laser induced breakdown in Argon at 0.35 m: theory and experiments" Phys. Rev. A 31 2300-2313.
8. Gamal, Y. EE- D. and Azzouz, I. M. (1987):"Influence of the collision frequency of the electron energy distribution function in laser – induced discharge in argon ".J. Phys. D: Appl. Phys. 20, 187 – 192.
9. Tsuda, N. and Yamada, J .(1997):"Observation of forward breakdown mechanism in high-pressure argon plasma produced by irradiation by an excimer laser".J. Appl. Phys. , 81(2),582-586.
10. Gamal, Y. EE-D. Omara, O. Ghazoulin, B. and Sabour, K. A. (1999) :"On the study of the electron Kinetic processes in the breakdown of argon by 0.53 m and 0.248 m Laser Radiation " .J. Phys. D: Appl. Phys. , 32,1633.
11. Davis, P. J. Smith, L. A. Giranda, C. and Squicciarini, M. (1991) : "Laser-induced plasma formation in Xe, Ar, N2, and O2 at the first four Nd:YAG harmonics".Applied Optics ,30 (30), 4358-4364.
12. Gamal Yosr E.E- D, I.M. Azzouz and M. El - Nady (1986):"Ruby laser induced breakdown of argon" Advances in laser science -1, Proceeding of AIP conference, December 1985 Dallas USA, No. 1, 46, page 578 – 9.
13. Rosen, D. I. and Weyl, I. G. (1987): "Laser-induced breakdown in nitrogen and the rare gases at 0.53 and 0.35 m".J . Phys . D: Appl.phys.,20 1264- 1276.
14. Grey Morgan C(1975): "Laser-induced breakdown of gases". Rep. Progr. Phys. 38, 621-65,

12/2/2012

f
o
r

0
.
3

<

<

2
0

e
V

The spatial analysis of effective factors on development of border rural regions of Saravan, Sib and Suran

Dr. Mohammad AjzaShokuhi [1], Dr.Barat Ali Khakpoor [2], Abdolsami Sepahi [3]

[1.] Assistant Professor, Department of Geography, Faculty of Letters and Humanities, International Branch of Ferdowsi University of Mashhad, Mashhad, Iran
[2.] Associate Professor,Department of Geography, Faculty of Letters and Humanities, International Branch of Ferdowsi University of Mashhad, Mashhad, Iran
[3.] Ph. D Student in Geography and Urban Planning, International Branch of Ferdowsi University of Mashhad, Mashhad, Iran
sepahi1387@yahoo.com

Abstract: Development planning in Iran in recent 50 years is mostly based on section strategies and is mostly without development views and attitudes based on development conditions. The study area as a sub system of Iran residencies system was not far from these problems and was encountering with some issues as low literacy and employment, the lack of good communication ways, the shortage of health facilities, low agriculture and industries level and etc. indeed, these conditions leads into structural balance and development level in the region. The current study aimed to recognize and evaluate the effective factors in the development of rural areas of Saravan tow n and field and library methods were applied both to evaluate the indices. To evaluate the development HDI and taxonomy were applied and for data analysis, the advanced statistical methods such as multivariate regression, factor analysis techniques were applied. The final results of this study showed that spatial distribution of development indices in the region was not balanced as environmental and geographical conditions and establishment of rural residencies had considerable effect in distribution of the indices and services. Also, the exact share of health factors variables, agriculture, border trading were the most effective factors in determining the development level of the region.
[Mohammad AjzaShokuhi, Barat Ali Khakpoor, Abdolsami Sepahi. **The spatial analysis of effective factors on development of border rural regions of Saravan, Sib and Suran.** *Life Sci J* 2012;9(4):4823-4829] (ISSN:1097-8135). http://www.lifesciencesite.com. 725

Keywords: Factor analysis; rural development; regression; Saravan; HDI

1. Introduction

The geographical environment has spatial and temporal dimensions consisting of different elements as city and village. Today, the villages are faced with different issues making consideration to the villages due to the dominance of systematic relations between effective factors in formation of geography space, city and village and the mutual effect of these two aspects.

Now, the villages are faced with some problems as the shortage of living space, low population and imbalanced distribution in various regions, sending migrants, the shortage of good employment, inadequate income, low education level, education, health, life expectancy, etc and the lack of regional balances and high difference between urban and rural facilities exacerbate these problems.

Therefore, until social and economical opportunities in rural regions are as today form, any attempt to solve it increases the regional inequalities, migration from villages and more problems in rural regions (Badri, 1989, 28). To reduce the inequalities, the planning should be used to balance between the population and environment facilities in the employment of each region and other effective factors in the development of each Geography region.

The villages in Iran are of great importance before land reformation. The rural economy in Iran is used to provide economical needs of the society and in terms of political issues, the destiny of Iran is with their destiny due to the dependency of Iran to villagers activities but after the advent of oil in Iran economy and the growth of single product economy, the real position of village was eliminated and positive rural economy of Iran was turned into a consumption economy. It seems that to achieve a prosperous economy out of dependency and single product economy, special attention to rural areas and planning rural problems is necessary.

The evaluation of rural development as make the planners informed with the most important capabilities and the lack of facilities of the regions is of great importance. As after the Islamic revolution, by the activity of Jihad in villages of Iran more services are predicted. It seems that in population stability in rural areas we didn't achieve positive results. Thus, despite considerable costs, there are many problems in rural areas and to investigate these issues and its reasons and services distribution we should do more studies. This study is carried out in this regard.

The strategic rural development is used to improve the rural life. It should consider all spatial elements and development indices and inside and outside effective factors (consulting engineers D.H.V, 1992, 20). What is considered of development is the full development of villages and in which all the systems are in mutual action and systematic attitude instead of section attitude should be considered. Thus, rural integrated development is dependent upon increasing production and variety of facilities and organizing spatial network of rural dispersed residential and defining the position of village in national development plans (Saeedi, 1998, 157). The mentioned processes like other rural regions affected rural areas of Saravan and Sib and Suran (Management and planning organization of Sistan Baluchistan, 2006, 6).

Of sum of the population of Saravan town in 1996, 58651 people were in urban areas and 109476 people in rural areas. Of the sum of the population of Saravan town in 1996, 58651 people were in urban areas and 109476 people were in rural areas and the urbanization rural people in this town was 34.01% and 65.99% that compared to the similar ratios in total province (46.2% urban, 53.88% rural), the high share of living in rural area and the importance of this living model in social composition of the population of the town is observed well (Plan and budget organization of sistan and Baluchistan, 1997, 40). The comparison of the employment composition in major parts of town showed that of sum of the employed people of the town 40.3% were in agriculture, 21% in industries and 38.2% in service activities. While the similar indices in total province were 33.1%, 22.7% and 44.2% (ibid, 4). It is considered that in the first degree, agriculture had the highest share in employment of this town that is done mostly in rural areas. Despite the high importance of agriculture in employment of the villages compared to other towns of the province in changes of urban and rural population of two recent period of statistics, it is observed that urban population growth rate of this town is two times more of similar rate of the province, while urban population growth rate for total province is 4.49%. This rate in Saravan was 9.46% and Saravan town had the highest urban population compared to other towns in the province. The above items show that migration from village to city in this town compared to other regions of province despite high potentials of rural areas was high. If this trend continues, not only the problems of urban regions are increased, but also, agricultural potentials of the villages related to employment are reduced. The current study was carried out in 14 towns of this city in 2007. To evaluate the development level of the towns, taxonomy and combinational development indices were applied. The applied indices in this study were used in accordance with statistics of 1996 and the comparison of the results with the statistics of 2006. The current study aimed to study the effective factors in development in human and natural aspects and by advanced techniques, the share of each of the factors in rural regions development of Saravan was defined exactly to find the final aim of achieving a good model of rural development in this town. The applied indices in this study were the combination of economical, social, etc items.

The rural regions of this town have the following issues:

1- Considerable migrant sending of the regional villages

2- The type of traditional agriculture despite the high capabilities of agriculture

3- The lack of adequate capital to do productive activities in the village

4- International and illegal activities

Based on the social, economical and political changes of Iran and formation of some issues as unduly development of cities population, increasing population, the increase of migration from rural areas to cities, the lack of national consideration to spatial structure of rural regions, the existence of the villages with low capability, geographical isolation and the weakness of communicative networks, low employment condition in villages as the characteristics of rural areas of most regions in the country and rural areas in the studied region. The following hypotheses and questions are raised:

1- The developments of border markets are effective in stability of the population in rural areas of Saravan town.

2- The cooperation companies of date product can create the most important aspect of employment in rural areas of Saravan town.

3- There is an association between development degree of rural areas and agricultural abilities.

Questions

1- Which are the employment capabilities in border villages of Saravan town?

2- Which are the effective measurements from the view of villagers of Saravan town to improve the development levels?

2. Research method

In this study, library, field and analytical methods were applied. In the first stage, the required resources were identified and by the existing statistics, the notes were taken and another part of the data of this study was provided by questionnaire, interview and direct observation.

In the second stage, the obtained data were classified and organized. For the analysis of spatial condition of developing the studied region, the data of statistics of 1996, 2006 were used.

In the third stage, the data were analyzed and after plotting the chart and map, factor analysis and HDI and taxonomy models were used. For exact calculation of the effective factors in the development, multi-variate regression was applied. In sum, the method is based on analytical-inductive method.

Data collection instruments

1- By referring to scientific centers as libraries and scientific centers, the theoretical and scientific resources were collected.

2- The required statistics were applied of the organizations and offices of Sistan and Baluchistan and planning management organization of the province.

3- The required maps were provided from survey organization (the map of Iran villages) and geography organization of army forces and by Iran statistics center data by Arc View software, it was done during the required plots.

Data analysis method

At first, the required data were entered by SPSS software and then the data analysis was done by statistics techniques of factor analysis and multivariate regression.

1- The analysis of the maps was done by Gis and Arc View software.

2- In the next stage, we investigated the condition of services in towns of the region and by showing the indices on the map, the existing condition of the indices was identified.

3- By factor analysis technique and multivariate regression in spss software to predict the development priorities model, the development index was considered as dependent variables and factor scores were considered as independent variable.

4- Finally by combining the factors, the conclusion and recommendations are presented.

The geographical condition of the study area

The study areas is Saravan, Sib and Suran in the east of Sistan and Baluchistan and geographically are in the same border from north and north west to Khash, west and south west to Iranshahr and Sarbaz and from east and south east to Pakistan. Saran is with the area of 13905 km2, Sib and Suran with the area of 6780 km2.

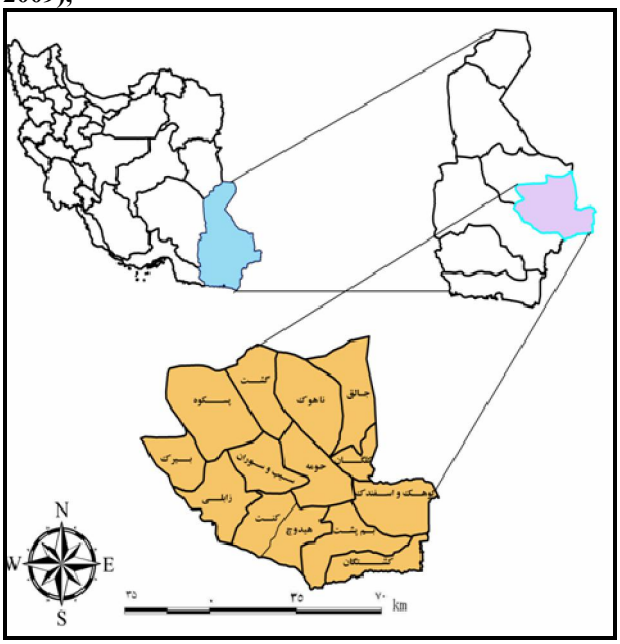
3. Filed indices

For better clarification of the condition of development of Saravan town villages and prioritizing the effective needs in the development based on the comments of the villagers themselves, a questionnaire with 28 questions was designed and was distributed by simple random method based on the population of each suburb, 30 samples were given. As the needs and facilities of rural regions of border suburb are different from other regions the study indices are divided into border suburb and non-border.

Table 1: The political divisions of Sistan and Baluchestan province, Saravan, sib and Suran

Locations	The number of towns	division	suburb	The number of cities	residential village
Sistan and Baluchistan	14	40	102	36	8908
Saravan town	----------	3	8	5	443
Sib and Suran	---------	2	4	2	264

Source. Management and planning organization, statistical journal of Sistan and Baluchistan, 2009),



Source. The authors

The study indices

1- Cooperative companies of producing date
2- Employment
3- Agriculture
4- Communication ways
5- The cooperative companies of agriculture
6- Border trading
7- Husbandry
8- Border market

The results of the study indices in border suburb

Based on Multiple Respose method in SPSS soft ware, the field interpretations were scored and the results are shown in Table 2.

Table 2: The scores of field indices of border suburb of Saravan towns

Number	Index / Dehestan	1	2	3	4	5	6	7	83
1	Bamposht	22	28	20	30	26	30	20	30
2	Keshtegan	21	27	19	29	25	29	19	29
3	Kuhak Esfandak	22	26	17	30	27	30	18	30
4	Jaleq	27	29	16	30	25	28	19	30
5	Kolegan	24	30	17	30	27	28	19	29
6	Nahug	25	29	27	29	30	27	18	28

After defining the scores of each suburb, the share of each index is defined by factor analysis. The investigation of the study variables in this part shows that totally two factors among the existing factors had the considerable effect on rural border regions of this town. The first factor 43.06%, second facto 29.31% variance is observed. In the first factor, the border trading indices, employment, cooperative companies of data production had the highest share achieving 95%, 75% and 64%, respectively. Table3. Also, Table 4 shows the factor scores of these indices.

Table 3: The variances of field indices of border suburb of Saravan town

First factor		Second factor	
Index	Variance percent	Index	Variance percent
Border trading	0.95	Agriculture	0.68
Employment	0.75	Communication ways	0.65
Border market	0.69	Husbandry	0.57
Date production cooperative companies	0.64		
Agriculture production cooperative companies	0.64		

Table 4: Factor scores of field indices of border suburb

Dehestan	Factor 1	Rank	Factor 2	Rank
Bamposht	-0.954	1	0.156	6
Keshtegan	-0.465	5	-0.471	5
Kuhak Esfandak	-1.015	3	-0.779	4
Jaleq	0.249	6	1.252	1
Kolegan	0.651	4	1.053	3
Nahug	1.534	2	-1.210	2

Source: The calculations of authors

Rural Development Index (RDI)

In this section, to achieve the better recognition of the development of the villages in the region and classification of the villages in terms of the classification development in three levels (supra development, middle development and sub-development) is done. In this study, HDI technique was used as a practical method but instead of using three required indices in the method of UN, 26 indices in economical, social, cultural, structural and agriculture are used.

Leveling the development of the studied villages by RDI

For hierarchical leveling of the villages of Saravan, RDIT technique was applied by which 26 indices in fourteen suburb of Saravan were selected in accordance with the statistics of 2003 and based on the results were analyzed.

The selected indices are as following:

1- The percent of total population of the villages
2- The percent of the literate population of total villages
3- The percent of the villages with water network
4- The percent of the villages with electricity
5- The percent of the villages with Asphalt way
6- The percent of the villages with health centers
7- The percent of the villages with health house
8- The percent of the villages with drugstore
9- The percent of the villages with physician
10- The percent of the villages with dentist
11- The percent of the villages with assistant nurse and midwifery
12- The percent of the villages with health assistant
13- The percent of the villages with social worker
14- The percent of the villages with telephone services
15- The percent of the villages with post mail services
16- The percent of the villages with agency services and post office
17- The percent of the villages with Islamic council
18- The percent of the villages with rural cooperative company
- The percent of the villages with elementary school
- The percent of the villages with guidance school
- The percent of the villages with high school
- The percent of human resources in agriculture sector of the villages
- The percent of the fields of the villages
- The percent of the deep well of the villages
- The percent of the tractor in villages

- The percent of the packing industries of data products in villages

Table 5: Determining the RDI of Saravan town based on the statistics of 2003

No.	suburb	Deprivation index	RDI	Final rank of rural development	Final rank of deprivation index
1	Bamposht	0.88	0.12	12	3
2	Keshtegan	0.70	0.30	5	10
3	Kuhak, Sfandak	0.70	0.30	6	9
4	Jaleq	0.88	0.12	12	3
5	Kalegan	0.90	0.10	13	2
6	Nahug	0.92	0.08	14	1
7	Birak	0.78	0.22	10	5
8	Zaboli	0.48	0.52	3	12
9	Paskuh	0.73	0.24	8	7
10	Sib and Suran	0.34	0.66	1	14
11	Humeh	0.52	0.48	4	11
12	Gasht	0.77	0.23	9	6
13	Kant	0.79	0.21	11	4
14	Hiduch	0.72	0.28	7	8
Mean			0.31		
SD			0.19		

Source. Authors' calculations

By investigating Table 5 and estimation of the development and rural deprivation of Saravan town by rural development technique, the suburb are classified into the following groups:

a. Supra development suburb

Sib, Suran and Zaboli suburb are in the first rank in terms of development levels and in terms of development index is higher than 52%. In terms of suburb rank, Sib and Suran are in the first position with the index 66%. Normally, the villages of this suburb are close to the center of suburb and in terms of national and economical facilities are in better position.

b. Mid-development suburb

The Dehestans that are between 12.50% in terms of development index are in this group and Humeh, Hiduch, Paskuh, Gasht, Birak, Kent, Keshtegan and Kuhak Esfandak are in this level.

c. Sub development suburb

Jaleq, Kalegan, Nahuk, Bamposht are lower than 12% in terms of development index and these suburb don't have natural, economical, etc facilities compared to other suburb.

The prediction model of development priorities of Saravan town based on multi variate regression model and factor analysis

By applying SPSS software and multivariate regression model, we can present the priority of developing rural regions based on factor scores. In these calculations, RDI is considered as dependent variable and factor scores are considered as independent variables.

The results of the calculations showed that R2 is the calculated determination coefficient as 95% and it shows that 95% of the development changes in the applied indices in its evaluation are related to these three factors. By reducing the percent of independent variables, determination coefficient is reduced the same and in case of its increase, the development of above regions are added. Table 6.

In Table 7, variance analysis calculates the significance of regression in linear relation between the variables with significance level Sig. 0.000 and significance at 98% is supported.

By Table 8 showing that the index in the regression model, the prediction model of development priorities of rural areas of Saravan town are presented. Thus, the regression prediction equation is calculated as following:

$Y=0/312+0/148F_1+0/109\ F_2+0/091\ F_2$

The significance level showed that the effects of first and second factor had the highest significance level, respectively. To predict the priorities of rural development of Saravan town was important because one unit change in standard deviation of development will change as 0.705, 0.520, 0.434 unit. Beta values show high importance of first factor compared to other factors. Thus, by considering regression model, we can say that prediction factors of development of the rural areas with bottom to up levels are:

1- Improving and increasing health services, development of communicative facilities (telephone, post office)

2- The development of elementary school, guidance school and high school, increasing the Asphalt communication lines

3- The development of date packing industry and additional activities, increasing the access to tractor

Table 6: The statistics of multivariate regression analysis of development indices of Saravan town suburb

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.977(a)	.955	.941	.05080

a Predictors: (Constant), REGR factor score 3 for analysis 1 , REGR factor score 2 for analysis 1 , REGR factor score 1 for analysis 1

Table 7: The variance analysis regression of multivariate regression analysis of development indices of Saravan town suburb

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.546	3	.182	70.563	.000(a)
	Residual	.026	10	.003		
	Total	.572	13			

a Predictors: (Constant), REGR factor score 3 for analysis 1 , REGR factor score 2 for analysis 1 , REGR factor score 1 for analysis 1

b Dependent Variable: RDI

Table 8: The coefficients of development regression indices of Saravan town suburb in the model

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.312	.014		22.953	.000
	REGR factor score 1 for analysis 1	.148	.014	.705	10.494	.000
	REGR factor score 2 for analysis 1	.109	.014	.520	7.737	.000
	REGR factor score 3 for analysis 1	.091	.014	.434	6.459	.000

a Dependent Variable: RDI

First hypothesis

The development of border markets affects population stability in rural areas of Saravan town.

In the investigation of field indices of border suburb as a need for population stability in the first and second degree of the development of suburb and the results showed that 69% of variance is located between the variables and it is in the first factor of the study. In addition, first factor includes 43.06% of society variance. Any change in facility of rural areas to the development of border markets affects directly on the development of the region and population stability compared to other factors. Thus, based on all the above items, we can say that first hypothesis is accepted and development of border markets affects the population stability in rural areas of Saravan town.

Second hypothesis

The cooperative companies of producing data are the most important aspect of employment in rural areas of Saravan town. Among the investigated indices in field studies, this index is consisting of 64% variance among border suburb. Among non-border suburb, it includes 89% of the variance. This shows that data production cooperative companies among rural areas of non-border of Saravan town are important to create job and the above hypothesis is supported.

Third hypothesis

There is an association between development degree of rural areas and agriculture abilities.

The loaded variables in the third factor have high correlation ranging 0.54-0.72% and it shows high share of agriculture in the rural development of Saravan town. In addition, in the prediction model, development priorities of Saravan town are achieved based on factor scores and rural development, the agricultural facilities are on the priority. This hypothesis is supported.

The investigation of research questions

First question

1- Which are the employment capabilities in border villages of Saravan town?

The results of field studies among border suburb of Saravan showed that among the raised questions in this field, border trading showed 95% of variance, development of border market 69% of variance and husbandry showed 57% of variance. It can be said that the most important employment capabilities in border rural regions of the studied area is border trading, development of border markets and husbandry.

Second question

2- What are the effective measurements from the view of villagers of Saravan town to improve development?

The results of analysis of the questions of this part by factor analysis technique showed that communicative ways development showed 0.96% variance, the development of cooperative companies of producing date with 0.89% variance, the development of cooperative companies of producing agriculture, 0.87% variance and increasing job showed 0.67% variance.

It can be concluded that the effective measurements by the villagers for development of these regions are 1- The development of communicative ways, 2- The development of cooperative companies of date production, 3- Development of cooperative companies of agriculture production, 4- increasing job.

4. Presenting the strategies and recommendations

The priority of needs is the initial principles in planning. The prediction of what improves the development level of each society is of great importance. By considering the findings of this study, the following recommendations are presented to improve the life condition and increasing development level in the villages of Saravan town:

1- First priority to increase the development level of the villages, improving the health facilities as this factor is one of the effective factors in all the indices.

2- The investigation of literacy factor in the region shows that this index is one of the infrastructural factors affecting all the development indices as directly and it is not in good condition. To improve the index condition by considering the position of each suburb, first priority is considering the villages located in Jaleq, Paskuh, Bamposht, Kuhak Esfandak, Gasht, Nahuk, Birak, Hiduch, Gashtegan, Kalegan and Kant. In improving the literacy condition the development of high school, guidance school and elementary schools with training specialized forces are recommended.

3- The third effective factor on the development of villages is improving agriculture condition. The results of this study showed that agriculture had considerable share on the development of the region. The improvement of this index is possible via investigating the capabilities of each region and exact identification of its abilities. The exact investigation of climatic conditions, region water and soil, controlling surface water is effective. Also, improving communicative ways are in the third priority.

4- The development of the facilities of access to drinking water, the development of communicative ways is in the next priority. Development of border markets in the region provides the background for stable employment and reduces the illegal activities in the region directly. By considering the priority of the needs and comments of villagers, the development of border market is recommended. The development of cooperative companies of producing date and agriculture production cooperative companies are the needs presented by people and its development is recommended.

5- To improve the life condition of people we can recommend another applied research based on the existing condition of each suburb as next researches of the researchers.

6- To develop date industry, we can propose the various stages of production, investment, export and marketing, etc as a study in continuing work to the students of this field.

7- The development of border markets in creating stable job as applied is another issue to continue this work that can be effective in development of the region.

8- To improve the index of education, absorbing the forces including native and non –native as long-term commitment as a special adoption via education can be the solution of problems of shortage of specialized force.

9- In health index as the most important and effective index in development of this region based on the findings of this study, we can absorb domestic forces of the province by increasing the percent of native acceptance in medical fields of total province.

Corresponding Author:
Abdolsami Sepahi
Department of Geography and Urban Planning
International Branch of Ferdowsi University of Mashhad, Mashhad
E-mail: sepahi1387@yahoo.com

References

1- Badri, Ali. 1991. Locating development of villages center, case study of Gazran of Qom, MA thesis, supervised by Dr. Mehdi Taleb. Tehran. Tarbiat Modares University.
2- Papli Yazdi, Hossein and Mohammad Amir Ebrahimi. 2006. Rural development theories, Tehran. SAMT publication.
3- Planning and budget organization. 1975. The evaluation of three-year performance of fifth plan. Tehran. Plan and Budget organization.
4- Plan and budget organization. 1986. Basic plan of monitoring Islamic Republic of Iran. Tehran. Plan and budget organization.
5- Planning and management organization of Sistan and Baluchistan. 2006. Statistical journal of 2005. Management and planning organization.
6- Planning and management organization of Sistan and Baluchistan. 2007. Statistical journal of 2005. Management and planning organization.
7- Saeedi, Abas. 1998. Rural Geography basics. Tehran. SAMT publication.
8- Soleimani tabar. Maryam. 2003. The investigation of spatial structure and analysis of rural development indices in Barsir town. MA thesis of Geography group of Tarbiat Moalem University

12/3/2012

Effect of feed additive "Exogenous Enzymes" on growth performance of Maghraby Camels

Adel E. M.[1] and H. EL-Metwaly[2]

[1]Department of Animal Production, Faculty of agriculture, Cairo University, 12613 Giza, Egypt.
[2]Camel Research Department, Animal Production Research Institute, Dokki, Giza, Egypt.
Akkb2010@gmail.com

Abstract: This experiment aimed to evaluate the effects of a mixture of exogenous enzymes (ZADO®) from anaerobic bacteria on growth performance, feed intake, nutrient digestibility and blood parameters. Eighteen growing Maghraby camels averaged, 268.83 kg body weight; 1.5-2 years. Camels were randomly divided into 3 equal groups (6 in each) of similar weight and age, which were offered complete rations with tow levels of ZADO® product. The first group was Zero g/h/d (control), the second group take 20 g/h/d of ZADO® and the third group 40 g/h/d, over a period of 90 days. Results indicated that 40 g supplementation showed the best response in DM and OM digestibility. Carbohydrate results showed significantly effects of ZADO® supplementation on crude fiber and Nitrogen free extract in R40 being, 78.23 %, 80.60% and R20 being, 75.56%, 77.23%, respectively with insignificant difference between R20 and C ration. NDF digestibility was significantly with R40 (75.77%) followed by R20 (72.99%) and C (71.17 %). Blood parameters of control and tested groups of camels were in normal range with slight decrease in total lipid. Total body gain and average daily gain (ADG) significantly differed among experimental groups being 61.87, 84.82 and 88.65 kg and 0.69, 0.94 and 0.98, in C, R20 and R40 kg, respectively. Data related to feed intake as DM, TDN showed insignificant difference among groups of camels. It could be concluded that growing male Maghraby camels fed on the diet containing ZADO® performed better than those offered the control ration. Moreover, adding ZADO® in camel ration (40g/h/d) was the better, as confirmed by the highest body weight gain, most of blood metabolites and digestibility.

[Adel E. M. and Hassan EL-Metwaly. **Effect of feed additive "Exogenous Enzymes" on growth performance of Maghraby Camels.** *Life Sci J* 2012;9(4):4837-4842] (ISSN:1097-8135). http://www.lifesciencesite.com.726

Keywords: Camel, ZADO®, digestibility, daily gain.

1. Introduction

The dromedary camel is one of the most important domestic animals in the arid and semi arid regions as it is equipped to produce high quality food at comparatively low costs under extremely harsh environments (Yagil, 1982; Yousif and Babiker, 1989). The role of the camel as a meat producer is becoming more important due to the versatile role it plays rather than as a symbol of social prestige, which was the role it used to play but which has since greatly diminished (Dawood and Alkanhal, 1995). The camel has great tolerance to high temperatures, high solar radiation and water scarcity. It can survive well on sandy terrain with poor vegetation and may chiefly consume feeds unutilized by other domestic species (Shalah, 1983). Tandon, Bissa, and Khanna (1988) noted that the camel is likely to produce animal protein at a comparatively low cost in the arid zones based on feeds and fodder that are generally not utilized by other domestic species due to either their size or food habits. Camel has a great capacity to produce meat. Now, camel has a more important situation as a meat-producing animal in Egypt (Mohmed *et al*., 2009).

In the same time, FAO statistics (FAO, 2010) refers to there are about 24.7 million camels in the World, of which 20.7 million are found in Africa, only 11 thousands from this numbers in Egypt. Also, numbers of camels decreased from year to another in Egypt (141 thousands in 2000, 120 thousands in 2005) according statistics and that contrasted with other countries.

A commercial exogenous enzyme mixture (ZADO®), prepared from anaerobic bacterium, has been shown to improve nutrient digestibility, as well as milk yield of cows fed diets containing Egyptian by-product feeds (Gado et al., 2007; Soliman, 2006), as well as live weight gain and feed conversion of sheep and goats fed wheat straw (Gado and Salem, 2008; Salem et al., 2007). Also, Gado *et al*. (2009) observed that the addition of enzymes increased intake of dry matter (DM) and organic matter (OM) was positively influenced by supplementation, and digestibility of all nutrients was higher in the total tract of supplemented cows with 40 g of ZADO®/h/ day, although the magnitude of the improvement varied among nutrients, with the highest improvement in NDF and ADF than the other nutrients.

The Egyptian Maghraby camel is medium in size with small but pointed hump. Besides pack use, the Maghrebi camel is used for all kinds of agricultural, industrial and draft purposes. A number of types are locally developed serve certain functions.

The Maghrebi camel generally responds to feeding and might gain about 700-1000 grams per day during the first year under intensive conditions (Wardeh, 2004). In the same time, there is a lack of information in the literature on using feed additives in the growing camel's rations. Therefore, the objective of the present study was to evaluate the effects of ZADO® supplementation of diets of Maghraby camel calves on feed intake, growth performance, nutrients digestibility and blood metabolites.

2. Material and Methods

Location

The present study was carried out at two places growth and digestion trial were take place at Camel Studies and Production Development Center in Matrouh governorate which belongs to Camel Research Department, Animal Production Research Institute, Agricultural Research Center and sample analysis and statistics at Animal production department, Faculty of Agriculture, Cairo University ,Giza, Egypt.

Experimental animals and rations

Eighteen growing Maghraby Camels averaged 268.83 kg body weight; 1.5-2 years old were divided into 3 groups of 6 in each according to live weight for 90 days trial. Animals in the control group C: were fed concentrate feed mixture (CFM) and clover hay plus rice straw without additives, while, in the tested rations animals were fed R20: C+ 20 g ZADO® and R40: C+ 40 g ZADO®.

Table: 1. Chemical composition and fiber fractions of ingredients in the basal ration.

Item	Feedstuffs			Ration
	CFM	Clover hay	Rice straw	
Chemical composition %				
DM	91.33	89.96	89.25	90.74
OM	89.21	90.00	86.33	88.97
Ash	10.79	10.00	13.67	11.03
CP	14.76	13.76	4.92	13.14
EE	3.08	1.28	1.03	2.41
CF	8.61	36.20	42.51	19.30
NFE	62.76	38.76	37.87	54.12
Fiber fractions %				
NDF	34.21	61.94	78.48	46.42
ADF	12.67	49.70	57.02	26.86
ADL	2.98	4.95	10.49	4.47
Cellulose	9.69	44.75	46.53	22.39
Hemi-cellulose	21.54	12.24	21.46	19.56

*CFM, concentrate feed mixture, NDF: neutral detergent fiber, ADF: acid detergent fiber and ADL: Acid detergent lignin

Digestion Trail

Digestive trail were conducted to determine the nutritive value of experimental rations. Each trial was divided into two stages: a preliminary 21-day period to allow the animals to adapt to each feed, and a 7-day experimental period during which voluntary feed intake was measured and total collection of feces. Feces samples were weighed and dried at 60°C for 24 hrs in a hot air oven. The dried samples of feces and feeds were ground to pass through 1mm sieve. Representative samples of feed and feces were stored in emerged bottles for chemical analysis. Meanwhile, the digestion coefficients and nutritive values of the experimental rations were calculated.

Chemical analysis

Feeds and feces were analyzed for proximate analyses (A.O.A.C., 1990). Nitrogen free extract was calculated by difference. Fiber fractions were analyzed according to Van Soest and Wine (1967) and the cellulose and hemicelluloses were calculated by difference.

Blood parameters

Blood samples were collected from camels at the end of digestion trail. The blood samples were taken from the jugular vein in dry clean glasses tubes using heparin as anticoagulant and then centrifuged for 15 minutes at 4000 rpm to obtain plasma. Biochemical of blood plasma constituents were determined by using commercial kits, total protein and creatinine as described by Tietz (1986 and 1990), albumin was determined according to Doumas *et al*., (1971), blood plasma urea was determined according to Patton and Grouch (1977). Alanin amino transferase (ALT) and activity of aspartate transfearse

(AST) were determined by the methods of Young (1990). Glucose (g/dl) was executed by using kits of Stanbio Laboratory Inc, procedure No. 1070. (San Antonio, Texas, USA). Total lipids, triglycerides and total Cholesterol (mg/dl) were quantified by using colorimetric method by using kits of Bio diagnostic company.

Statistical analysis

Data were analyzed using the general liner model procedure of SAS (2000). One way ANOVA procedure was used to analyze the feed intake, digestibility, growth performance and blood parameter data following the next model; $y_{ij} = \mu + T_{ij} + E_{ij}$,were: μ is the overall mean of $y_{ij;}$ T_{ij} is the treatments effect; E_{ij} is the experimental error. The differences among means were separated according to Duncan's New Multiple Range Test (Duncan's 1955).

3. Results and discussion

Digestion coefficients and nutritive values

Data concerning nutrients digestibility and nutritive values are presented in Table (2). The results indicated that dry matter and organic matter digestibility was significantly lower (P<0.05) with control ration (C, 71.85%, 74.31) than rations with ZADO® supplementation with insignificant differences between R20 and R40 (73.34%, 75.87 and 76.56, 79.10 %). Also, data refers to 40 g supplementation gives best results in DM and OM digestibility, same trend reported by Gado *et al.* (2009). ZADO® addition did not appear any significant (P<0.05) effect on crude protein and ether extract digestibility. Carbohydrate results showed significantly effects of ZADO® supplementation on crude fiber and Nitrogen free extract in R40 being, 78.23 %, 80.60% and R20 being, 75.56%, 77.23%, respectively with insignificant difference between R20 and C ration. There were insignificant (P<0.05) differences in results related to fiber fractions digestibility among control and experimented groups in ADF, cellulose and hemicelluloses digestibility. But NDF digestibility was significantly with R40 (75.77%) followed by R20 (72.99%) and C (71.17 %). Exogenous enzyme in ZADO® product, rich in xylanolytic, cellulase, α-amylase and protease activity, had positive effects on digestion of NDF in TMR, agreed with Krause et al. (1998), who suggested that enzymes can improve nutrient degradation in high concentrate diets. Perhaps the net effects of fibrolytic enzyme mixtures are not limited to the dietary component to which the enzymes are applied, which may explain why fibrolytic enzymes can be effective in improving digestibility of the non-fiber carbohydrates in addition to increasing digestibility of fiber when enzymes are added to the concentrate portion of a diet, or to high-concentrate diets (Beauchemin *et al*., 2003).

The nutritive values of tested rations presented in Table (2) indicated that the TDN of experimental rations were significantly differ 68.25, 69.90 and 72.79% with C, R20 and R40, respectively. The corresponding values of digestible crude protein were 9.09, 9.21and 9.73 % for rations C, R20 and R40 with significant difference of R40 than other groups. In this study, this is in harmony with Kholif (2008) when goats fed on rumen content with 20 g ZADO® addition.

Table 2. Effect of ZADO® additive on digestion coefficients and nutritive values

Item	Experimental rations			±SE
	C	R20	R40	
Digestibility, %				
DM	71.85b	73.34ab	76.56a	1.23
OM	74.31b	75.87ab	79.10a	1.13
CP	69.16	70.06	74.07	1.47
EE	75.84	79.50	79.94	1.47
CF	73.14b	75.56ab	78.23a	1.20
NFE	75.63b	77.23ab	80.60a	1.23
NDF	71.17b	72.99ab	75.77a	1.17
ADF	62.66	63.38	68.48	1.86
Cellulose	68.02	68.41	74.16	1.71
Hemicelluloses	82.84	85.11	85.78	1.28
Nutritive values, %				
TDN	68.25b	69.90ab	72.79a	1.01
DCP	9.09b	9.21b	9.73a	0.19

Means in the same row with different superscript are significantly different (P<0.05).

Blood parameters

Results concerning the effect of feeding camel on ZADO® product on some blood parameters are shown in Table (3). The average values of plasma total proteins of the experimental groups were 9.21, 9.35 and 9.01 g/dl for C, R20 and R40, respectively. Plasma albumin and globulin concentration in the showed similar trend of total plasma proteins, which indicated slight variations among tested groups of camel. Al-Busadah (2007) investigated that, total protein and albumin in different breeds of camels ranged from 4.9-10.2 g/dl and 3.1-6.2 g/dl. Triglycerides values showed significantly (P<0.05) effect of ZADO® addition, R40 had a higher value (111.51) than R20 (104.84) and C (79.27). In the same context, Mohamed, (2008) found that triglycerides in Egyptian camels ranged from 0.71 to 1.02 mmol/l. On contrast, total lipid data appeared lower significant values with R40 (873.22) than R20 and control ration. In the same trend, Nazifi *et al*., 2000 noticed that concentration of lipid in camels less than 6 years old 3.19-4.18 g/l.

Blood glucose concentration was highly significant with R20 (57.97) than other experimental rations with insignificant effect between C and R40. In this area, Bhatia (1986) reported the range 75-120 mg/dl and concluded that concentration of glucose in the blood of camels is generally higher than that in other ruminants. Urea and creatinine concentration in blood plasma was insignificantly (P<0.05) with control ration and supplemented rations. Chiericato et al. (1986a) found that urea in male camel may be up to 39.9 g/dl. Also, Patodkar *et al*., (2010) reported that adult male and female blood creatinine being; 1.87 and 2.37 mg/dl.

There was significant difference (P<0.05) in blood AST concentration among camels fed on C , R20 and R40 ration being, 69.05, 77.24 and 59.11 IU/L, respectively. In this respect, Mohamed and Hussein (1999) showed that AST concentration ranged between 34 – 148 IU/l. On the other side, the values of ALT ranged from 6.86 to 7.68 IU/L without significant differences among groups. Aichouni *et al.,* (2010) stated that the ALT content in blood of different camel breeds were 3.01 – 6.91 IU/l. Also, Sarwar and Majeed (1997) reported that serum ALT activity was positively correlated with serum globulin and total protein levels. Blood plasma transaminase enzymes activity (ALT and AST) are the most important indicators of liver cells activity where increasing the concentration of these enzymes indicate that the tissue activity are destroyed (Clifton Blincoe and Dye, 1958).

Table 3. Effect of ZADO® additive on blood parameters of camel fed the experimental rations.

Item	Treatments			±SE
	C	R20	R40	
Total proteins, g/dl	9.21	9.35	9.01	0.15
Albumin, g/dl	5.58	5.41	5.74	0.18
Globulin, g/dl	3.63	3.94	3.27	0.23
Triglyceride, mg/dl	79.27b	104.84ab	111.51a	9.12
Total lipid, mg/dl	900.00ab	932.79a	873.22b	15.73
Glucose, mg/dl	38.11b	57.97a	55.31ab	5.84
Urea, mg/dl	34.85	42.25	26.34	7.64
Creatinine, mg/dl	0.97	1.08	1.09	0.05
AST, IU/L	69.05ab	77.24a	59.11b	4.92
ALT, IU/L	6.86	7.68	6.87	0.47

Means in the same row with different superscript are significantly different (P<0.05).

Growth performance

Daily gain, feed intake and feed conversion of camels fed two levels of additives and control group were measured. The results of live body weight values are shown in Table (4). There were insignificant differences (P<0.05) among the camels at the beginning of the experiment, being; 268.37, 286.62 and 269.50 kg. Final body weight gain was slight significant between supplemented groups (353.45, 358.15 kg with R20 and R40, respectively) and control group (330.25 kg) without insignificant differences between R20 and R40. In the same context, total body gain and average daily gain (ADG) significantly differed among experimental groups being 61.87, 84.82 and 88.65 kg and 0.69, 0.94 and 0.98, in C, R20 and R40 kg, respectively. Nutritive values of the experimental rations as TDN (68.25, 69.90 and 72.79 %) and DCP (9.09, 9.21. and 9.37 %), significantly differed, which may elaborate the former results concerning total or daily gain in difference. Daily growth rates for camels vary widely between regions, breeds and within the same breed. The limited work carried out on improving camel nutrition demonstrated significant relationships between daily gain and daily intake of concentrates for dromedary camels. The present data agreed with Wardeh, (2004), who recorded that Maghreby camel generally responds to feeding and might gain about

700-1000 grams per day during the first year under intensive conditions. Growing Maghraby camel which fed two different rations (control ration and Nigella sativa ration) at 3% of body weight for 98 day that ADG was 886 – 950 g/d respectively (Mohamed, 2007).

So, this study appeared when camel fed on good quality feeds and improved rumen ecosystem by feed additives for instance exogenous enzyme gives the best results with that promise animal. In this respect, Kamoun (1993) reported that ADG of Magraby dromedary camels kept on pastures of the Mediterranean type were 760 g up to 5 months of age, 605 up to 10 months of age and 353g from 12 up to 18 month of age. Also, Kamoun, 1995 investigated that, camels fed a diet with high dietary protein and energy gained more weight (550 g/d) than non-supplemented camels fed only on mangroves (260 g/d). A commercial exogenous enzymemixture (ZADO®), prepared from anaerobic bacterium, has been shown to improve live weight gain and feed conversion of wheat straw in sheep and goats (Gado and Salem, 2008; Salem et al., 2007).

Data related to feed intake as DM, TDN showed insignificant difference among groups of camels. However, DCP intake was significantly lower with control group (54.41 g) than experimental groups (57.29 and 58.71 g in R20 and R40). On the other hand, results of feed conversion indicated that supplementation of ZADO® in camel ration gives best results with feed conversion as DM (8.68 g DM/g gain). Consistent with El-Badawi and Yacout (1999) found that, camels and steers fed concentrate mixture (14%CP) at level 2% of body weight and rice straw *ad lib* showed ADG 810g and 770g for camels and steers respectively. On contrast, feed conversion (kg DM/kg gain) was nearly similar for both species (10.01 and 10.76 kg for camels and steers respectively).

Table 4. Average live body weight, feed intake and feed conversion of growing camels fed ZADO® additive.

Item		Experimental rations			±SE
		C	R20	R40	
Body weight change	Initial live body weight, kg	268.37a	268.62a	269.50a	14.31
	Final live body weight, kg	330.25b	353.45a	358.15a	14.51
	Total live weight gain, kg	61.87b	84.82ab	88.65a	7.38
	Average daily gain, kg	0.69b	0.94ab	0.98a	0.08
Feed intake, h/d. As fed, kg	CFM	4.29	4.46	4.50	---
	CH	1.40	1.46	1.47	---
	RS	0.95	0.99	1.00	---
Feed intake, h/d. on DM basis	DM, kg.	5.99	6.22	6.27	0.57
	TDN, kg	4.08	4.34	4.56	0.57
	DCP, g.	54.41b	57.29a	58.81a	1.52
Feed conversion (g feed/ g gain)	DM, g.	8.68a	6.62b	6.39b	2.49
	TDN, g.	5.19	4.62	4.65	0.59
	DCP intake, g.	0.8	0.6	0.6	0.06

Means in the same row with different superscript are significantly different (P<0.05).

Conclusion

It could be concluded that the exogenous enzyme product (ZADO®), sourced from anaerobic bacterium and added to the camel rations, increased daily gain due to enhanced nutrient intake, and nutrient digestibility, as well as increased feed conversation.

Corresponding author
Adel E. M.
Department of Animal Production, Faculty of agriculture, Cairo University, 12613 Giza, Egypt.
Akkb2010@gmail.com

References

1. A.O.A.C. (1990). Official methods of an analysis. 15th Ed. Association of Official Analytical Chemists. Washington, DC., USA .
2. Aichouni, A.; Jeblawi, R.; Dellal, A.; Hammou, H. and Aggad, H. (2010). Breed variation in blood constituents of the one-humped camel (Camelus dromedaries) in Algeria. J. Camelid Sci. 3:19-25
3. Al-Busadah, Kh. A. (2007). Some Biochemical and Haematological Indices in Different Breeds of Camels in Saudi Arabia. Scientific J. of King Faisal Uni. (Basic and Applied Sciences),(1): 14-28
4. Beauchemin, K.A., Yang, W.Z., Morgavi, D.P., Ghorbani, G.R., Kautz, W., Leedle, J.A.Z., 2003. Effects of bacterial direct-fed microbials and yeast on site and extent of digestion, blood chemistry, and subclinical ruminal acidosis in feedlot cattle. J. Ani.Sci. 81: 1628-1640.

5. Bhatia, J. S. 1986. Special aspects of physiology of digestion in camel. Farm Animals 1: 15-20.
6. Chiericato, J. M.; Warfa, A. A. and M. P. Schiappelli, (1986a). Influence of sex of the dromedary on some constituents of the blood. Rivista di Zootecniae Veterinaria 4: 196-199. (Veterinary Bulletin 57: 3890).
7. Clifton Blincoe and W. B. Dye (1958).Serum Transaminase in White Muscle Disease. J. Ani. Sci., 17:224-226.
8. Dawood, A., & Alkanhal, M. A. (1995). Nutrient composition of Najdi- Camel Meat. Meat Science, 39:71-78.
9. Doumas, B.; Wabson, W. and Biggs, H. (1971). Albumin standards and measurements of serum with bromochresol green. Clin. Chem. Acta., 31(1):87-96.
10. Duncan, D. B. (1955). Multiple Range and Multiple F Test. Biometrcs, 11:10.
11. El-Badawi, A. Y. and Yacout, M. H. M. (1999). Comparative study on growth performance of camel (Camelus dromedaries) calves and cattle steers in the feedlot system. Proceeding of the 7 th Sci. conf. on Anim. Nutr. (ruminant, poultry and fish). 19- 21 Oct., 1999, El-Arish, Egypt. Part 1: Egyption J. Nutr. And Feeds, 1999, 2: Special Issue, 319- 330.
12. FAO, Food and agriculture Organization of the United Nation Rome (2010). Animal Live Statistics.
13. Gado, H.M., A.Z.M. Salem, P.H. Robinson, M. Hassan., (2009). Influence of exogenous enzymes on nutrient digestibility, extent of ruminal fermentation as well as milk production and composition in dairy cows. Animal Feed Science and Technology 154 (2009) 36–46eed Science and Technology 154: 36–46
14. Gado, H.M., Metwally, H.M., Soliman, H., Basiony, A.Z.L., El Galil, E.R., (2007). Enzymatic treatments of bagasse by different sources of cellulase enzymes. In: The 11th Conf. Animal Nutr., Al-Aqsor-Aswan, Egypt on 2 November, 13–18, vol. 10, p. 607.
15. Gado, H.M., Salem, A.Z.M., (2008). Influence of exogenous enzymes from anaerobic source on growth performance, digestibility, ruminal fermentation and blood metabolites in lambs fed of orange pulp silage in total mixed ration. In: 59th Annual Meeting of the European Association for Animal Production, Vilnius, Lithuania, August 24–27, p. 228 (Abstract).
16. Kamoun, M. (1993). Reproduction and production of Maghrabi dromedaries kept on pastures of the Mediterranean type. Etudes et Synthesrs de l`lEMVT. (1993), no. 41, 117-130; Actes de l`Atelier "Peut on ameliorer les performances de reproduction des camelins" Paris, France, 10-12 September.
17. Kamoun, M. (1995). Dromedary meat: production, qualitative aspects and acceptability for transformation. Option Mediterraneennes Serie B, Etudes et Recherches, 13, 105–130.
18. Kholif, A. E., (2008). Use of Biotechnology to Improve the Utilization of rumen contents in ruminants ration. Ms.C., Fac. of Agric., Ain Shams Univ.
19. Krause, M., Beauchemin, K.A., Rode, L.M., Farr, B.I., Nǿrgaard, P., (1998). Fibrolytic enzyme treatment of barley grain and source of forage in high-grain diets fed to growing cattle. J. Ani. Sci. 76: 2912-2920.
20. Mohamed, H.A and Hussein, A.N. (1999). Studies on normal haematological and serum biochemical values of the ' Hijin' racing camels (Camelus dromedarius) in Kuwait. Vet. Res. Commun. Jun; 23(4):241-8.
21. Mohamed, H.E. (2008). Factors Affecting the Plasma Lipid Status in Camels (Camelus dromedarius). Res. J. Biol. Sci., 3 (4): 444-445
22. Mohamed, M. I. (2007). Evaluation of Growth Performance for Growing Maghraby Camel Fed on Un-conventional Feed. Int. J. Agri. Biol., Vol. 9, No. 1, p 18-21.
23. Mohamed, M.I., Y.A. Maareck, Soha S. Abdel-Magid, I.M. Awadalla, (2009). Feed intake, digestibility, rumen fermentation and growth performance of camels fed diets supplemented with a yeast culture or zinc bacitracin. Ani. Feed Sci. and Tech., 149 : 341–345.
24. Nazifi, S.; Gheirsari, H.R.; Abbasali Poorkabir, M. and Saadatfar, S. (2000). Serum Lipids and Lipoproteins in Clinically Healthy Male Camels (Camelus dromedarius) Vet. Res. Communi. 24: 527-531.
25. Patodkar, V.R.; Somkuwar, A.P.; Parekar, S. and Khade, N. (2010). Influence of sex on certain biochemichal parameters in nomadic camels (camelus dromedarius) nearby pune, in Maharashtra. Vet. World. 3:115-117.
26. Patton, F. G. and Grouch, S. R. (1977). Colorimetric determination of urea, Anal. Chem. 49, 468.
27. Salem, A.Z.M., El-Adawy, M.M., Gado, H., Khalil, M.S.M., 2007. Feed intake, nutrient digestibility and animal growth performance in sheep and goats fed wheat straw. ADSA PSA AMPA ASAS Joint Annual Meeting, San Antonio, TX, USA, July 8–12. J. Anim. Sci. 85 (Suppl. 1), 107 (Abstract).
28. Sarwar, A. and Majeed, M. A. (1997). Interrelationships between 30 parameters of blood in normal one-humped camel in summer. J. of Camel Prac. And Res., 4:1, 35-39.
29. SAS (2000). SAS users guide Statistical analysis system inistitute, Inc., Cary, Nc, USA.
30. Shalah, M. R. (1983). The role of camels in overcoming world meat shortage. Egyptian J. of Vet. Sci., 20:101-110.
31. Soliman, M.S., (2006). Utilization of peanut hay in ruminant feeding. Ph.D. Thesis. Alexandria University, Alexandria, Egypt.
32. Tandon, S. N., Bissa, U. K., and Khanna, N. D. (1988). Camel meat: Present status and future prospects. Annals of Arid Zone, 27: 23–28.
33. Tietz, N. W. (1986). Text Book of Clinical Chemistry. W. B. Saunders, Philadelphia, 1271.
34. Tietz, N. W. (1990). Clinical Guide to Laboratory Tests 2nd Ed. Philadelphia.
35. Van Soest, P. J. and Win, R. M. (1967). Use of detergent in the analysis of fibrous feed. IV. Determination of plant cell wall constituent. J. Assoc. Off. Anal. Chem. 50:50-55.
36. Van Soest, P. J. and Win, R.M. (1967). Use of detergent in the analysis of fibrous feed. IV. Determination of plant cell wall constituent. J. Assoc. Off. Anal. Chem. 50:50-55.
37. Wardeh, M.F. (2004).Classification of the dromedary camels. J. Camel Sci. vol (1)1-7.
38. Yagil, R. (1982). Camels and camel milk. FAO Animal Production and Health. Publications Division, Food and Agriculture Organization of the United Nations. Via delle Terme di Caracalla, 00100 Rome, Italy (No. 26).
39. Young, D. S. (1990). Effects of drugs on clinical laboratory testes, 3rd Ed. 3:6.
40. Yousif, O. K. and Babiker, S. A. (1989). The desert camel as meat animals. Meat Science, 26, 245-254.
41. Yousif, O. K., & Babiker, S. A. (1989). The desert camel as meat animals. Meat Science, 26, 245-254.
42. Zakaria Farah, Matthias Mollet, Mario Younan and Ragge Dahir, (2007). Camel dairy in Somalia: Limiting factors and development potential. Livestock Science 110: 187–191

12/2/2012

The selection of the most adaptable line of Carthamus tinctorius L. to the stress of non-irrigation conditions in mild region (Khoramabad, Lorestan)

Hadis Zaremanesh [1], Mohammad Hasanvand [2], Hossein Sabzi [2]

[1.] Department of agriculture, Payame Noor University, Iran
[2.] Researchers at the Agricultural Research Center of Lorestan, Iran
hadis_zaremanesh@yahoo.com

Abstract: To perform research project of Carthamus tinctorius L. non-irrigation was selected as the most adaptable one of Carthamus tinctorius L. under non-irrigation conditions in mild-cold regions of 19 lines in the form of completely random blocks in fall 2010 in research stations of Sarab Changayi located in km 5 of Khoram Abad road in three replications. The results showed that there was a significant difference between the numbers for the attributes height, number of grains in the bush, oil percent, the number of grains in the tray and the number of trays in the bush at level 1% and for the attributes of the weight of 1000 grains and grain performance per hectare at level 5%. The results of the comparison of the average attributes of the study showed that line 12 (306599 PI) in attributes of 1000 grains weight, height, the number of grains in the bush, oil percent, the number of grains in the tray, the number of tray in the bush and grain performance per hectare had the highest performance to other lines. Line 4 with 32g had the maximum weight of 1000 grains among the investigated lines. In the division to main components, the main component was named as the number of grains in the bush, the second component as height and the third component as biological performance.

[Hadis Zaremanesh, Mohammad Hasanvand, Hossein Sabzi. **The selection of the most adaptable line of Carthamus tinctorius L. to the stress of non-irrigation conditions in mild region (Khoramabad, Lorestan).** *Life Sci J* 2012;9(4):4836-4840] (ISSN:1097-8135). http://www.lifesciencesite.com . 727

Keywords: Carthamus tinctorius L.; non-irrigation; line; adaptation; stress

1. Introduction

Carthamus tinctorius L. is a plant with deep root with thorny leaves and these two attributes created the toleration of dryness and heat (26). Carthamus tinctorius L. is cultivated in arid and hot areas as an oil grain, birds grain, paint of the flowers or medicinal applications (23). In three decades, this plant was used as oil grain and its oil grain is ranging from 20 to 45%. Middle East, namely Iran is one of the variety centers of this plant. Carthamus tinctorius L. is a good plant to be cultivated in Mediterranean weather. Any factor stopping the natural metabolic stages of a plant is considered stress. Dryness stress is one of the most important environmental stresses restricting the performance of cultivation plants. Hashemi Dezfuli in the investigation of the effects of dryness stress on Carthamus tinctorius L. showed that leaf surface, plant height, the number of branches and the number of bolls were reduced due to draught and despite the reduction of dry matter of stem and root was increased to the root to the stem. To modify the dryness-resistant plants, some of the researchers believed in selecting the genotypes in good conditions (non-stress)(13, 29) and some other people emphasized on the selection in stress conditions (16,. 30). Most of the researchers recommended the selection of genotypes in both conditions of stress and non –stress. Sinemna et al. found that the potential of performance in stress conditions was not considered as the best criterion resistant to dryness and the stability of performance in stress and non-stress conditions are good criteria for the reaction of genotypes to humidity stress. The selection of genotypes in stress and non-stress environments caused the congestion of good points and genotypes with high performance. Rosbel and Hamblin called the difference between the performance of a genotype in stress and non-stress conditions as Tolerance (TOL) and introduced as dryness tolerance index. High value of this index showed the relative sensitivity of genotypes to stress, thus low values of TOL are good. The selected genotype based on this index has low relative performance in non-stress conditions but in stress conditions have high performance. Productivity mean index (MP) was provided by these two researchers that is defined as mean sum of the performance of a genotype in stress and non –stress conditions. Fernandez proposed Stress Tolerance Index (STL) as a criterion to select draught tolerant figures. The high value of STI showed high tolerance of stress and high potential performance. The figures with high STI are the genotypes with high stress and non –stress conditions. Another index being proposed by Fernandez is mean productivity geometrics (GMP). This index has high power compared to MP in genotype separation. Stress Susceptibility Index (SSI) was proposed by Fisher and More, low amount of SSI showed the low changes of performance in stress conditions to the non-stress

conditions and more stability of genotype. Richard believed that selecting the genotypes in stress and non-stress conditions of dryness caused the congestion of good Alels and the high performance genotypes are selected. Patil et al evaluated seven varieties of Carthamus tinctorius L. in five regions of non – irrigation and 8 varieties in 4 regions of irrigation (without humid stress). There was significant difference in terms of the performance of grain among the genotypes and genotype X of the environment. In this test, good genotypes were defined for each of irrigation and non-irrigation conditions. Abolhasani in the investigation of 15 local lines of Carthamus tinctorius L. in stress and non-stress conditions of draught showed that draught stress had negative influence on the weight of grain and the attributed of the number of grains in boll in stress conditions 71% and in non-stress conditions 70% of the changes of performance of grain in the bush were justified.jamshid Moqadam and Pordad with the review of 15 Iranian and foreign genotypes of Carthamus tinctorius L. under humid stress condition in controlled conditions and stated that in stress conditions of 0.4, 0.8 mega Pascal, the length of the root of genotypes is increased and by reduction of the growth of the stem, had more sensitivity to the root. Most of the Iranian genotypes in sprouting stage had better reaction to foreign genotypes in stress conditions. In humid stress conditions, one of the plant parts that is firstly damaged is plasma membrane as permeability of cell membrane is increased and cause that the existing electrolytes inside the cell infiltrate to outside the cell. One of the most important strategies in the modification of increasing the resistance to dryness is that cell membrane after being faced with water stress, keeps its stability and is not disintegrated. Various tests are applied to measure cell membrane stability (GMS) that can define the resistance to dryness among the plants. In most of the methods, a genotype is faced in two different conditions (dryness stress and control) and it is measured by a specific method (measuring EC), the amount of electrolytes is measured by which genotype is infiltrated in stress and control conditions. By comparing these two types, we can find which genotypes in stress conditions could keep the cell membrane better and fewer electrolytes infiltrated of it. Kuchva and Jorjif in the evaluation of resistance to dryness of climate figures, observed less destruction in resistant figures cell membrane to dryness. Based on the results of the tests, this is raised that free perolin make the membrane stable during dryness stress period. The stability of cell membrane under humid stress was reported as the main component of tolerance to dryness. The damage to cell membrane by the dryness is evaluated via measuring electrolicich of the cells (cell infiltration). Fokar et al reported that reduction of grain weight in each cluster and percent of damage to cell membrane had high negative correlation (r=0.97**). Vinslo and Smirov showed that the genotypes tolerating dryness stress, had less cytoplasm membrane destruction. Kuchva and Jorjiof by dryness stress via PEG6000 solution and submerging the root of two varieties of barley in this solution showed that relative content of the water of the leaves was reduced in stress conditions and by increasing stress stability was decreased. This investigation was done to investigate the resistance to dryness in genotypes of Carthamus tinctorius L. and identification of resistant genotypes by plot and disintegration to main components of the lines resistant to stress with high grain performance and they were in future modification plan.

2. Materials and methods

Initial operation of providing land including sow, disk was prepared and based on the results of soil test, fertilization (phosphor and potash) was done, the test was done as total random blocks with three replications and the lines were cultivated randomly in each block. The cultivation time was 89.9.3 and greening 89.10.5 and investigation time as 4.18 to 90.4.21. During cultivation season, some attributes as the start and ending of flowering, flowering period length, bush height, the number of trays in the bush, the number of grains in the tray were written. After harvesting, the grain performance was determined in the plot and per hectare. It was done by MSTATC, Minitab, Excel software and they were analyzed by the comparison of the averages by Duncan method. The division to main components was applied by Minitab software and score plat method was used to identify and compare resistant lines to non-irrigation conditions stress.

Principle components analysis

The aim of most of the multi-variant statistical methods is summarizing the attributes as by some quantities, the population can be distinguished. This method was proposed by Pearson in 1901 and by Hotling its calculation method was recommended.

In principle components analysis, the first component had the maximum changes and after than the maximum variance was related to the second component and the last component had the least variance. This multi-variant analysis was used to identify the important attributes, the reduction of the volume of data and grouping of the figures and stress-resistant genotypes are used.

3. Results

The results showed that there was significant difference between the figures in attributes of height, the number of grains in the bush, oil percent, the number of grains in the tray and the number of trays in the bush in level 1% and for the attributes of the

weight of 1000 grains and grain performance per hectare at level 5%. The results of the comparison of the average of the attributes showed that line 12 (306599PI) in the attributes of the weight of 1000 grains, height, the number of grains in the bush, oil percent, the number of grains in the tray, the number of trays in the bush and grain performance per hectare had maximum performance to other lines. The maximum biologic performance was related to line 7 (253541PI|) and the minimum biological performance was related to line 12 (306599PI). The low biological performance of line 12 showed that the plant to increase the performance required taking high energy to produce the grain and due to this fact, grain performance was with biological performance reduction. Line 12 with the height of 117cm had maximum height and line 19 with 93cm had lowest height. Leaf surface index was related to line 5 with 23cm and at minimum state was for line 12 with 13cm. Lines 2, 4, 7, 11, 12, 14 had the maximum weight of 1000 grains. In principle components analysis the first component 0.463 as the component of the number of grains in the bush, the second component with 0.445 as height component and third component with 0.753 were named biological performance. The results showed that lines 12, 7, 8, 2, 17 with high performance is weak to non-irrigation conditions stress. Liens 15, 19, 13, 6, 3 as with no high performance showed relative resistance against the stress of non-irrigation condition.

The average of squares of the attributes						
Changes resources	Degree of freedom	The number of grain in the bush	OIL (%)	The performance of grain per Hectare (Kg)	The number of grain in the tray	The number of grain in the bush
Block	2	30391	611.46	118710	1375.8	28.077
Treatment	18	12085**	243.16**	71784*	547.1**	11.165**
Error	36	1833	36.88	76805	82.99	1.694
Total	56					
C.V		22.3	6.54	17.39	19.1	11.54
*,** were significant at 5%, 1%						

The average of squares of the attributes						
Changes resources	Degree of freedom	The weight of 1000 grains gr	Biological performance gr	Grain performance in plot gr	Height (cm)	LAI (cm)
Block	2	12.754	4728202	24009	36.02	46.89
Treatment	18	10.54*	2117802	14534	158.72**	28.62
Error	36	5.632	2691798	15548	40.68	45.12
Total	56					
C.V		19.41	24.61	14.93	13.87	12.22
*,** were significant at 5%, 1%						

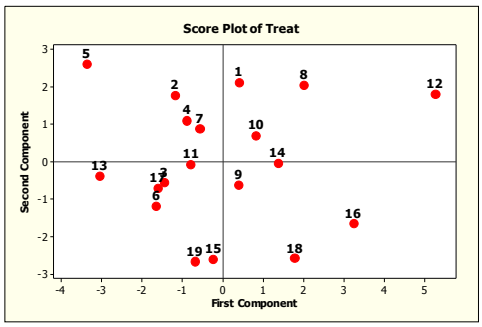
Line	The comparison of the average of the study attributes for 19 lines							
	The weight of 1000 Grains gr		Biological Performance gr		Height (cm)		LAI (cm)	
1	29.67	ab	4316.67	ab	102.67	b	19.18	ab
2	31.67	a	3716.67	b	107	ab	15.35	b
3	28	b	5100	ab	102	b	12.7	cd
4	32	a	3150	bc	108.33	ab	16.39	bc
5	28.67	b	3200	bc	114	ab	22.5	a
6	25.67	b	3433.33	bc	100	b	14.18	c
7	30.33	a	5850	a	112.33	ab	18.94	ab
8	29	ab	3500	bc	110.33	ab	15.92	bc
9	26.67	b	3483.33	bc	106	ab	11.57	cd
10	27	b	3166.67	c	110	ab	17.87	ab
11	31.67	a	4550	ab	101	ab	12.58	c
12	30	a	3300	c	117	a	11.28	cd
13	27	b	4100	b	108.33	ab	18.16	ab
14	28.67	a	3733.33	b	111	ab	10.74	cd
15	26.67	b	5166.67	ab	99.33	b	14.14	cd
16	26.67	b	4683.33	ab	99.33	b	16.4	ab
17	28	ab	5166.67	ab	90.33	b	16.83	ab
18	28.33	ab	5116.67	ab	97	b	13.47	cd
19	27.67	ab	4483.33	ab	92.33	c	16.51	ab

Line	The comparison of the average of the studied attributes									
	The number of grain in bush		Oil percent		Performance of grain per hectare (Kg/h)		The number of grain in tray		The number of tray in bush	
1	211.33	c	29.98	b	929.33	a	44.96	b	6.42	ab
2	138	def	19.57	cd	861.33	ab	29.36	b	4.19	bc
3	120.33	cde	17.07	def	776.67	b	25.6	b	3.66	cde

4	134.33	cde	19.05	cd	695.33	c	28.58	ab	4.08	bc
5	86.67	e	12.29	ef	655	b	18.44	c	2.63	ef
6	106.33	cde	15.08	cde	667.67	c	22.62	cd	3.23	cde
7	161.33	bc	22.88	bcd	767	ab	34.33	b	4.9	b
8	244	b	34.61	ab	823.33	ab	51.91	ab	7.42	ab
9	164.67	bc	23.36	bcd	642	c	35.04	b	5.01	b
10	196.33	c	27.85	b	684.67	c	41.77	ab	5.97	ab
11	135.67	cde	19.24	cd	713	ac	28.87	b	4.12	bc
12	324.67	a	41.05	a	956	a	69.08	a	9.87	a
13	69	d-f	9.79	e	708.67	ac	14.68	cde	2.1	f
14	191.67	c	27.19	b	624.33	b	40.78	ab	5.83	ab
15	141.33	cde	20.05	cd	491.33	c	30.07	b	4.3	bc
16	262.67	b	37.26	ab	464.33	cd	55.89	b	7.98	ab
17	133.33	cde	18.91	cd	809.33	ab	28.37	b	4.05	bc
18	202	c	28.65	b	412.67	de	42.98	ab	6.14	ab
19	131	cde	18.58	cd	464.67	cd	27.87	b	3.98	bc

Principal components analysis of the attributes

Attributes	First component	Second component	Third component
1000 grains	0.015	0.345	0.356
Biological performance	-0.041	-0.306	0.753
Grain performance in plot	-0.201	0.5	0.172
Height	0.09	0.445	-0.367
LAI	-0.223	0.214	0.266
The number of grains in bush	0.463	0.103	0.101
Oil percent	0.463	0.103	0.101
Grain performance per hectare	-0.201	0.5	0.172
The number of grains in tray	0.463	0.103	0.101
The number of trays in bush	0.463	0.103	0.101



The chart of score plot of the lines to identify and compare the figures resistant to stress

Corresponding Author:
Hadis Zaremanesh
Tehran
Iran
E-mail: hadis_zaremanesh@yahoo.com

References

1. Abolhasani, Kh. 2002. The evaluation of the lines of local masses of Carthamus tinctorius L. in two humid regimes. MA thesis of vegetable modification. Agriculture school. Industrial University of Isfahan.
2. Ashkani, J. H., Pakniat. 2004. The genetic evaluation of quantity indices of dryness-resistent in spring Carthamus tinctorius L. The abstract of the articles of 8th congress of cultivation and modification of Iran vegetable. Rasht. Iran.
3. Aminian, R. Khodambashi, Emami, M. Yadegari. 2006. The investigation of correlation between various attributes and dryness-resistant indices in Normal bean. The abstract of the articles of 8th congress of cultivation and modification of Iran vegetable. Pardis Abureihan. Tehran University.
4. Pordad, S. S. 2006. Carthamus tinctorius L. (by Li Daju, H.H. Mandel). Sepehr publications.
5. Pourmosavi, S. M. M. Geloy, J. Daneshian. 2006. The evaluation of animal fertilizer on the stability of cell membrane and chlorophyll of soybean in dryness stress conditions. The abstract of the articles of 8th congress of cultivation and modification of Iran vegetable. Pardis Abureihan. Tehran University.
6. Tarinejad, A. 1998. The evaluation of reaction of the lines of local masses of fall wheat to water conditions and water shortage stress. The abstract of the articles of 8th congress of cultivation and modification of Iran vegetable. Karaj. Iran.
7. Eshqi, A. Gh, H. Kazemi. M. Valizadeh. H. Lesani. 1998. Using physiological methods in evaluation of germ plasmas resisent to cold weather. The abstract of the articles of 8th congress of cultivation and modification of Iran vegetable. Karaj. Iran.
8. Azizinejad. R. 2004. The investigation of stability analysis and polymorphism by RAPD-PCR in 16

genotypes of Carthamus tinctorius L. under the humid stress in controlled conditions and farm. Sciences and techniques of agriculture and natural resources. 10(2): 155-168.
9. Daneshian, J. S. Qalebi, P. Jonubi. 2006. The evaluation of the coefficient of response of performance and sensitivity indices and tolerance to dryness in figures. The abstract of the articles of the 9th congress of cultivation sciences and modification of Iran vegetables. 5 to 7 of Shahrivar 2006. Pardis Abureihan. Tehran University.
10. Bandurska h.2000.does proline accumulated in leaves of water stressed barley plants confine cell membrane injury? i.free proline accumulation and membrane injury index in drought and osmotically stressed plants. Acta physiologiae plantarum 22:409-415.
11. Betran, F.J., d. beck, m. Benziger and g.o.edmeades.2003. genetic analysis of inbred and hybrid grain yield under stress and nonstress environments in tropical maize. Crop sci.43:807-817.
12. Bewly , l.d.1979. Physiological aspects of desiccation tolerance. Ann. Rev. plant physiol.30:195-238.
13. Blum, a. and A.ebercon.1980. call membrane stability as a measure of drought and heat tolerance in wheat. Crop sci. 21:43-47.
14. Ceccarelli, S.Grando. 1991. Selection environment and environmental sensitivity in barley. Euphytica 57:157-167.
15. Clavel, d.2005. analysis of early variation in responses to drought of groundnut(Arachis hypogea L.)For using as breeding traits. Environ. Exp.bot.54:219-230.
16. Farshadfar, e. and j. shutka.2003. multivariate analysis of drought tolerance in wheat substitution lines. Cereal res. Commune. 31(1,2):33-40.
17. Fernandez, g.c.j.1992.effective selection criteria for assessing plant stress tolerance. In: proceeding of a symposium, 13-18 Aug., Taiwan.
18. Fisher ,R.A. and R.Maurrer. 1978. Drought resistance in spring wheat cultivars. i. grain yield responses. Aust.J.Agri. res.30:801.
19. Fokar, m., a. Blum. And h.t. nguyen. 1998. Heat tolerance in spring wheat. ii grain filling. Euphitca. 104:9-15.
20. Hashemi Dezfouli, a. 1994. Growth and yields of safflower as affected by drought stress. Cop res. Hisar.7(3):313-319.
21. Knowles, p.f.1969. centers of plant diversity and conservation of germplasm: safflower. Econ. Bot. 23:324-329.
22. Kocheva, k. and g. Georgive. 2003. Evaluation of the reaction of two contrasting Barly (Hordeum vuldar l.) cultivars in response to osmotic stress with PEG6000. BLUG.J.Plant physiol. 290-294.
23. Levitt, j.1980. responses of plant to environmental stresses vol.ii. water, radiation , salt and other stresses. academic press., new York.
24. Mundel, H.H., H.C.Huang, G.C.Kozub and D.J.S.Barr. 1995. Effect of soil moisture and temperature on seedling emergence and incidence of incidence of pythium damping-off in safflower (Carthamus tinctorius L.). can.j. plant sci.75:505-509.
25. Patil, P.S., A. M.patil and A.B Deokar. 1992. Stability of yield in rain fed and irrigated safflower. J. Maharashtra agric. Univ.17:66-69.
26. Pourdad, S.S. and a.beg. 2003.sanfflower: a suitable oilseed crop for dryland areas of Iran. In: proceeding of 7th international conference on development of dry lands. Sep.14-17, Tehran, Iran.
27. Rajaram, s. and m.van Ginkle. 2001. Mexico 50 years of international wheat breeding. Pp.579-604. In: Bonjean , A.P., angus, W.J.(eds.), the word wheat book: a history of wheat breeding. Lavoisier pub., Paris, France
28. Rathjen, a.j.1994. the biological basis of genotype× environment interaction: its definition and management. Proceeding of the seventh assembly of the wheat breeding society of Australia, Adelaide, Australia.
29. Richarde , R.A. 1996. Defining selection criteria to improve yield under drought. Plant growth reg.20:157-166.
30. Rosielle, A.A. and j. Hamblin. 1981. Theoretical aspect of selection for yield in stress and non-stress environments. Crop sci.21:943-946.
31. Saba, J., M.Moghaddam. K.Ghassemi and M.R. Nishabouri. 2001. Genetic properties of drought resistance indices.J.Agric. Sci.Technol.(JAST).3:43-49.
32. Sinmena, b., p.c. Struik , M.M.Nachit and J.M.Peacock. 1993. Ontogenetic analysis of yield components and yield stability of durum wheat inwater- limited environments. Euphytica 71:21-219.
33. Sio-Se Mardeh, A., A.Ahmadi, K.Poustini and V.Mohammadi. 2006.evaluation of drought resistance indices under various environmental conditions. field crop res. 98:222-229.
34. Venkateswarlu, B.and k Ramesh.1993.cell membrane stability and biochemical response of cultured cells of groundnut under polyethylene glycol- induced water stress. Plant sci. 90:179-185.
35. Winslowe, m.d. and n. Smirnoff. 1984. Techniques used to breeders nurseries of drought resistance. Botany 3:45-46.
36. Yau, S.K. 2005 yield increase of barley following safflower sunflower in a cool, semi – arid Mediterranean area. in: Proceeding of 6th international safflower conference. June 6-10, Istanbul, turkey.

9/6/2012

Genetic Diversity among Some Tilapia species Based on ISSR Markers

Y.M. Saad[1,2], M.A. Rashed[3], A.H. Atta[3], and N.E. Ahmed[3]

[1]Dept. of Biol. Science, Fac. of Sciences, King Abdulaziz Univ., KSA.
[2] Genetic Lab., National Institute of Oceanography and Fisheries (NIOF), Egypt.
[3] Dept. of Genetics, Faculty of Agric., Ain Shams Univ., Shobra El-Kheima, Egypt.
yasser_saad19@yahoo.com

Abstract: Inter-simple sequence repeat (ISSR) analysis was used to develop fish species-specific molecular markers for four Tilapia species (*Oreochromis niloticus, Oreochromis aureus, Tilapia zillii and Sarotherodon galilaeus*). 12 ISSR primers were tested to assess the effectiveness of ISSR analysis in discriminating among the four applied fish species. Some ISSR markers were detected as species-specific for *O. niloticus*, *O. aureus*, *S. galilaeus* and *T. zillii* species. The Phylogenetic relationships among applied fish species were reconstructed using deferent methods (Sokal & Sneath, Dice and Simple match coefficients). The percentages of polymorphism were ranged from 0% to 67% within *S. galilaeus*, while polymorphism values were ranged from 0% to 100% in the other studied fish species. The highest genetic dissimilarity value was observed between *O. aureus* and *T.zillii*. In contrary, the lowest dissimilarity value was observed between *O.niloticus* and *O. aureus*. ISSR analysis was an attractive tool for species identification. These markers were recommending when coupled with appropriate statistical analyses in fish species identification and classification.

[Y.M. Saad, M.A. Rashed, A.H. Atta and N.E. Ahmed. **Genetic Diversity among Some Tilapia species Based on ISSR Markers.** *Life Sci J* 2012;9(4):4841-4846] (ISSN:1097-8135). http://www.lifesciencesite.com. 728

Keywords: Tilapia, Genetics, ISSR, Identification and Dissimilarity.

1. Introduction

Genetic markers are fundamental tools for monitoring fish populations (Rashed *et al.,* 2008) and fish species genetic variability (Saad *et al*., 2009). Genetic markers should be conducted to provide the information needed for a sound management of farming fish (such as Tilapia fish) and wild fish stocks (Saad *et al*., 2009). This way will be useful, especially in fish breeding programs which use genetic markers as marker-assisted selection to improve the fish performance and/or economic traits (Rashed *et al*., 2009).

Tilapias have become the most important fish species for freshwater aquaculture in tropics and subtropics areas because of the relative ease of culture in variety of aquaculture systems and their favorable attributes as fish food (EL-Tawil, 1984; Ladewig and Shwantes, 1984; Bezrukov, 1987 and Rashed *et al*., 1998).

Inter-simple sequence repeat (ISSR) as a molecular technique does not require the knowledge of flanking sequences (Zietkiewicz *et al*. 1994). This technique uses a single primer containing the repetitive sequence of a microsatellite. The amplified DNA segments include the nucleotide sequence situated between two microsatellites blocks, yielding a multilocus marker system useful for genetic diversity analysis (Maltagliati *et al.,* 2006). The variable lengths of these amplified DNA sequences allow for the identification of differences between closely related species, although they reveal little information about the genetic variability within a single species (Reddy *et al.,* 2002).

ISSR analysis has been successfully applied in fish gene tagging (Ammiraju *et al.,* 2001), variety fingerprinting or genetic diversity analysis (Archak *et al*., 2003). This analysis is widely used for studying the genetic background of some plants (Blair *et al*., 1999), some animal such (Kol and Lazebny, 2006) and fish such as *Paralichthys* sp. (liu *et al.,* 2006).

The present study aims to assess the molecular genetic variability among four Tilapia species (*O. niloticus, O. aureus, T. zillii* and *S. galilaeus*) using ISSR analysis. In addition this technique was used to detect some Tilapia species-specific DNA markers. ISSR markers will be useful, especially in Tilapia breeding programs which use genetic markers as marker-assisted selection to improve the Tilapia performance and/or economic traits.

2. Material and Methods

Tilapia zillii (Z), *Sarotherodon galilaeus* (G), *Oreochromis niloticus* (N) and *Oreochromis aureus* (A) species individuals were collected based on their morphological characterization (Trewavas, 1983) from an Egyptian fish farm (National Institute of Oceanography and fisheries, EL-Qanatair EL-Khairia station, Egypt). Ten fish individuals were sampled from each collected tilapia species.

From each specimen, approximately 1 x 1 cm of caudal fin tissue was excised, placed in a 70 %

isopropanol and held at 4°C for subsequent DNA extraction. DNA extraction and purification were performed according to (Hills *et al*., 1996).

Twelve ISSR primers (Table 1) were originally selected (Biotechnology Laboratory, University of British Columbia, primer set 9) to measure the genetic variability among applied Tilapia species.

PCR reaction was prepared in a 10 µl contained a 50 ng of DNA, a 0.3 µM of primer, a 0.2 mM of dNTPs, a 25 mM of $MgCl_2$, a 0.5 unit of Taq polymerase and a 1 X buffer. PCR program was consisted of one cycle for 2 min. at 94°C, 30 cycles for (30 sec. at 94°C, 45 sec. at 44°C & 1.5 min. at 72°C) and one cycle for 10 min. at 72°C.

Gel images were analyzed using *GelAnalyzer3* software to determine molecular sizes, presence (1) or absence (0) in addition, frequencies and polymorphism type of the amplified fragments, the mean of band frequency and the polymorphism percentage for each primer were calculated.

Data analysis:

Data were analyzed as reported by Rashed et al., (2011). *NTSYSpc2.01b* and *SPSS* (10, and 15) software were used to estimate the similarity percentages between the four Tilapia species and reconstructing the phylogenetic relationships using Sokal & Sneath, Dice and Simple match coefficients (Dice, 1945, Nei and Li 1979 and Rohlf 1997 .

3.Results

ISSR analysis was successfully used to examine the genetic polymorphism, to detect species-specific ISSR markers and to determine the variability among the four applied Tilapia species.

The Molecular sizes (bp) of the specific ISSR markers for the four applied Tilapia species were presented in Table (2). *O. niloticus* species had eight specific ISSR markers at molecular size (MS) ranged from 1560 bp (814A primer) to 240 bp (HB10 primer). *O. aureus* species had eight specific ISSR markers at MS ranged from 1500 bp (814A primer) to 390 bp (HB13 primer). In addition, *S. galilaeus* species had ten specific ISSR markers at MS ranged from 1450 bp (814A primer) to 420 bp (HB11 primer). Finally, *T. zillii* had five specific ISSR markers at MS ranged from 1350 bp (814A primer) to 340 bp (HB15 primer).

A total of 2426 detected bands were produced (628, 603, 638 and 557 bands were produced in the species of *O. niloticus*, *O. aureus*, *S. galilaeus* and *T. zillii*, respectively). The number of bands with different molecular sizes which amplified by the 12 tested ISSR primers were ranged from 10 (HB12 in *T. zillii*) to three (HB13 in *O. niloticus* and *O. aureus* and HB15 in *S. galilaeus*, *O. niloticus* and *O. aureus*) with the mean of six bands per primer. The numbers of detected ISSR bands were varied among the four studied tilapia species. Bands were ranged from eight to three bands for *O. niloticus*, *O. aureus* and *S. galilaeus* species and from ten to four bands for *T. zillii* species (Table 3).

Table (1): ISSR primer names and sequences

Code	Sequence	Code	Sequence
814A	5' $[CT]_8$TG 3'	17899A	5' $[CA]_6$AG 3'
844A	5' $[CT]_8$AC 3'	HB10	5' $[GA]_6$CC 3'
844B	5' $[CT]_8$GC 3'	HB11	5' $[GT]_6$CC 3'
17898A	5' $[CA]_6$AC 3'	HB12	5' $[CAC]_3$GC 3'
17898B	5' $[CA]_6$GT 3'	HB13	5' $[GAG]_3$GC 3'
HB14	5' $[CTC]_3$GC 3'	HB15	5' $[GTG]_3$GC 3'

Table 2. The Molecular sizes (bp) of the specific ISSR markers for the four studied tilapia species.

O. niloticus		*O. aureus*		*S. galilaeus*		*T. zillii*	
Primer	bp	Primer	bp	Primer	bp	Primer	bp
814A	1560	814A	1500	814A	1450	814A	1350
844A	590	844A	770	814A	730	814A	690
844A	460	844A	550	844A	710	844A	1530
17898A	450	844A	410	844B	870	HB11	940
17898A	330	844B	540	844B	500	HB15	340
HB10	240	17898A	410	17898A	940		
HB11	1090	HB10	500	HB10	1010		
HB13	340	HB13	390	HB11	420		
				HB14	770		
				HB14	470		

* N, A, G & Z refer to *O. niloticus*, *O. aureus*, *S. galilaeus* and *T. zillii*, respectively.

The highest polymorphism percentages were detected in *T. zillii*. Within this species, five ISSR primers (844B, 17898A, 17898B, 17899A and HB14) generated a 100% of polymorphism (Table 3).

Table (3): Average of band frequencies (A.B.F), polymorphism percentages (P%) and number of detected ISSR bands (n) within each studied tilapia species.

Primer code	*O. niloticus*			*O. aureus*			*S. galilaeus*			*T. zillii*		
	A.B.F.	P%	n	A.B.F.	P%	n	A.B.F.	P%	n	A.B.F.	P%	n
814A	0.86	20	5	0.84	20	5	1.00	0	5	0.92	20	5
844A	0.96	20	5	0.80	40	4	0.83	33	6	1.00	0	5
844B	0.90	40	5	0.84	60	5	0.98	17	6	0.65	100	4
17898A	1.00	0	4	1.00	0	4	0.88	20	5	0.80	100	4
17898B	1.00	0	5	1.00	0	4	0.98	20	5	0.68	100	6
17899A	0.95	25	8	0.95	13	8	1.00	0	4	0.80	100	5
HB10	0.95	13	8	0.82	44	8	0.96	33	8	0.82	56	9
HB11	0.97	17	6	0.98	17	6	0.97	29	7	0.90	20	5
HB12	0.90	100	7	0.87	100	7	0.96	13	8	0.74	80	10
HB13	1.00	100	3	1.00	100	3	0.74	60	5	0.83	50	4
HB14	0.99	14	7	0.99	14	7	0.93	29	7	0.86	100	8
HB15	1.00	0	3	1.00	0	3	0.73	67	3	0.68	75	4
Mean ± Se	0.96 ± 0.01	29 ± 10	6 ± 0.5	0.92 ± 0.02	34 ± 10	5 ± 0.5	0.91 ± 0.03	27 ± 5.9	6 ± 0.45	0.81 ± 0.03	67 ± 11	6 ± 0.6

The Similarity mean and standard error (Se) values were calculated within the studied Tilapia species based on the 12 ISSR markers via the three similarity coefficients Table (4). Relatively, the lowest values were detected within *T. zillii.*

Table (4) Similarity mean and standard error (Se) values within the studied tilapia species based on the 12 ISSR markers via the three similarity coefficients.

Tilapia species	Dice (Nei and Li)		Simple matching		Sokal and Sneath I	
	Mean	Se	Mean	Se	Mean	Se
O. niloticus	0.961	0.004	0.966	0.003	0.982	0.002
O. aureus	0.984	0.004	0.955	0.003	0.977	0.002
S. galilaeus	0.951	0.004	0.955	0.003	0.977	0.002
T. zillii	0.830	0.019	0.875	0.012	0.931	0.007

The highest genetic dissimilarity value was detected between *O. aureus* and *T.zillii* species. In contrary, the lowest dissimilarity value was determined between *O.niloticus* and *O. aureus* species (Table 5). The dendrogram (Figure1) showed the genetic relationships among the four applied Tilapia species based on ISSR analysis. However, the lowest genetic distance was noticed between *O. niloticus* and *O. aureus* species.

Table (5): Similarity values among the studied tilapia species based on the 12 ISSR markers via the three similarity coefficients.

	Dice (Nei and Li)	Simple matching	Sokal and Sneath I
O. niloticus & *O. aureus*	0.666	0.704	0.827
O. niloticus & *S. galilaeus*	0.496	0.541	0.702
O. niloticus & *T. zillii*	0.354	0.450	0.620
O. aureus & *S. galilaeus*	0.471	0.527	0.691
O. aureus & *T. zillii*	0.324	0.436	0.607
S. galilaeus & *T. zillii*	0.435	0.514	0.679

Comparing the results of SPSS10 and NTSYSpc2.01b, we found that the relationships between *O. niloticus* and *O. aureus* are slightly differed among the three used similarity coefficient and this appears in the NTSYSpc2.01b dendrograms but not appeared in the SPSS10 dendrograms where the same distance between *O. niloticus* and *O. aureus* although the different similarity measures. Also, the

distance between the combined cluster of *O. niloticus*, *O. aureus* and *S. galilaeus* and *T. zillii* reflects the similarity between the four species in the case of NTSYSpc2.01b dendrogram but in the SPSS10 the dendrogram showed *T. zillii* distantly related from other applied fish species (Fig. 1). The same results were found using SPSS10, SPSS13 and SPSS15 versions. We suggest the use of NTSYSpc2.01b to deduce the phylogenetic relationships.

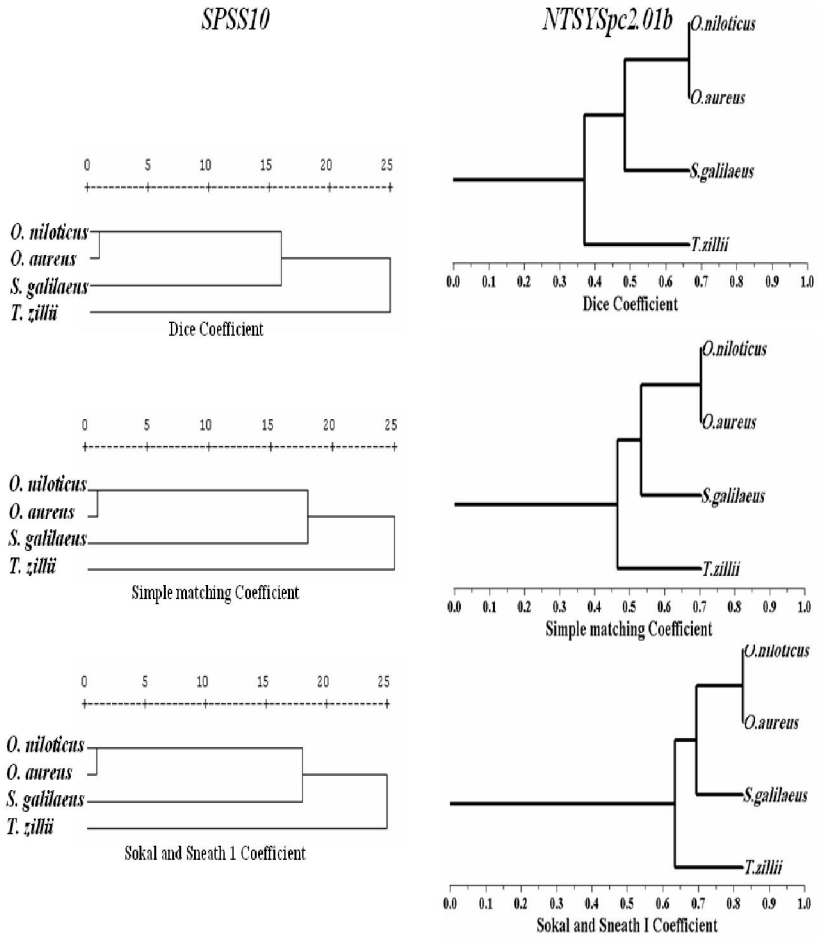


Figure 1. Dendrograms represent the comparison of *SPSS10* and *NTSYSpc2.01b* via three similarity coefficients in assessing the phylogenetic relationships using ISSR markers

4.Discussion

ISSR technique was used because it is simple and reliable tool for assessing the molecular genetic variability within and among many living organisms with highly reproducible results and abundant polymorphism (Kol and Lazebny, 2006 and Lalhruaitluanga and Prasad, 2009). Moreover, the potential applications of ISSR analysis for diverse aims is depend on the variety and frequencies of microsatellites within the specific genomes. (Chunjiang *et al*., 2005). In addition, variable ISSR patterns have potentials as dominant markers for studying genetic diversity of many fishes (Tong *et al*., 2005).

In the present study, ISSR analysis was offered some species-specific markers. The numbers of these molecular markers were varied from species to species. These DNA markers will be useful value, especially in fish breeding programs, which use genetic markers as marker-assisted selection to improve the fish performance (Rashed *et al.,* 2009).

The same idea was tested by Rashed *et al*., (2011). They used RAPD marker to detect the genetic variations among some tilapia species. They found that, the values of similarity among the four studied Tilapia species were high in Sokal and Sneath I, moderately in Simple matching and the low in Dice due to the use of shared present and absent fragments between each two estimated Tilapia species.

The molecular genetic markers are widely used to identify lines or strains, define stock diversity, monitor inbreeding, diagnose simply inherited traits

and even to improve stocks (Rashed *et al*., 2008 and 2009). The application of DNA-based genetic analysis as marker-assisted selection in fish research (such as tilapia) and stock development and management is still not fully maximized (Kocher *et al*., 1998 and Rashed *et al*., 2009).

ISSR analysis of the genetic diversity among the four applied Tilapia species showed that, the lowest genetic distance was noticed between *O. niloticus* and *O. aureus*. This conclusion was previously confirmed using another analysis such as RAPD. However, Saad *et al*. (2002) used bulked segregate analysis to reconstruct the phylogenetic relationships among three tilapia species (*O. niloticus, O. aureus* and *T. zillii*). They found that *T. zillii* species was distantly related from both *O.aureus* and *O.niloticus* species.

Liu *et al*., (2006) studied the genetic diversity in three *Paralichthys olivaceus* populations using ISSR analysis, which was confirmed to be a reproducible and sensitive tool for the study of population genetics of these fish. The genetic variability of domestic hatchery populations has implications to the conservation of natural *Paralichthys olivaceus* resources (Yun-Guo *et al.,* 2006).

The use of ISSR primers consisting of degenerate anchors or degenerate motifs increased the number of amplified markers. Since ISSR analysis is an easy to perform, high flow-through technique may represent it an alternative for the RAPD, better reproducibility was characterized due to the elevated annealing temperatures.

An especially attractive feature of ISSR analysis is its flexibility in terms of experimental design, where the number of generated amplicons may be optimized by changing the number of the core repeat units and anchoring bases (Liu and Wendel, 2001).

We suggest that ISSR analysis should be a standby choice for genome mapping or gene tagging and marker-assisted selection. For its high simplicity, ISSR analysis should be the first choice for genome mapping or gene tagging for organisms (which genomic knowledge is limited).

The above-mentioned exploitation and further studies would be significant for the basic and applied research on fisheries & aquaculture genetics and extend the knowledge of microsatellite conservation and evolution in Tilapia fish.

Corresponding author
Y.M. Saad[1&2]
[1]Dept. of Biol. Science, Fac. of Sciences, King Abdulaziz Univ., KSA.
[2] Genetic Lab., National Institute of Oceanography and Fisheries (NIOF), Egypt.
yasser_saad19@yahoo.com

References

1. Ammiraju JSS, Dholakia BB, Santra DK, Singh H, Lagu MD, Tamhankar SA, Dhaliwal HS, Rao VS, Gupta VS, and Ranjekar PK, 2001. Identification of inter simple sequencebrepeat (ISSR) markers associated with seed size in wheat. Theor. Appl. Genet., 102: 726-732.
2. Archak S, Gaikwad AB, Gautam D, Rao EVVB, Swamy KRM, and Karihaloo JL 2003. Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, and AFLP)for genetic analysis of cashew (*Anacardium occidentale* L.) accessions of India. Genome, 46: 362–369.
3. Bezrukov,V.F. 1987. Esterase isozymes and their variation in grass Carp Tsitologiya . Genetika, 21:293-297.
4. Blair MW, Panuad O, Mc Couch SR 1999. Inter simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and finger printing in rice (*Oryza sativa* L.). Theor. Appl. Genet., 98: 780-792.
5. Chunjiang Y., Zhanwang Y., Fanna k., Suowei W. and Bin W. 2005. R-ISSR as a New Tool for Genomic Fingerprinting, Mapping, and Gene Tagging. *Plant Molecular Biology Reporter*, 23: 167–177.
6. Dice, L.R. (1945). Measures of the amount of ecologic association between species. Ecology, 26: 297-302. Cited after Rohlf 1997.
7. El-Tawil, M.Y. (1984). Distribution of lactate dehydrogenase isozyme patterns of gobioid fishes. BULL.-MAR.-RES.-CENT.,-TRIPOLI. . 5: 5-35.
8. Hillis, D. M., B. K. Mable, A. Larson, S. K. Davis and E. A. Zimmer 1996. Nucleic acids IV: Sequencing and cloing, In: D.M. Hillis, C. Moritz, B. Mable (Eds.), Molecular systematics 2nd edn., pp. 342-343. Sunderland, Massachusetts: Sinauer Associates, Inc.
9. Kocher, T.D.; W. J. Lee; H. Sobolewska; D.Penman and B.Mc-Andrew 1998. A Genetic linkage map of a cichlid fish, the tilapia (*Oreochromis niloticus*). Genetics, 148: 1225-1232.
10. Kol L. and Lazebny O. 2006. Polymorphism of ISSR–PCR Markers in Tuvinian Population of Reindeer *Rangifer tarandus., Russian Journal of Genetic.*, (42)12: 1464–1466.
11. Ladewig, P. and L.L. Schwantes 1984. Loci that encode the lactate dehydrogenase in 23 species of fish belonging to the orders Cypriniformes, Siluriformes and Perciformes: Adaptative features. COMP.-BIOCHEM.-PHYSIOL., 77B. (4): 867-876.
12. Lalhruaitluanga, H. and M.N.V. Prasad 2009. Comparative results of RAPD and ISSR

markers for genetic diversity assessment in *Melocanna baccifera* Roxb. growing in Mizoram State of India. Afr. J. Biotechnol., 8(22): 6053-6062.

13. Liu, B. and J.F. Wendel , 2001. Inter simple sequence repeat (ISSR) polymorphisms as a genetic marker system in cotton. Mol Ecol Notes, 1: 205-208.
15. Liua, Y.G.; S.L. Chena; J. Lia and B.F. Lib., 2006. Genetic diversity in three Japanese flounder (*Paralichthys olivaceus*) populations revealed by ISSR markers. Aquaculture, 255(1-4): 565-572.
16. Maltagliati F., Lai T., Casu M., Valdesalici S. and Castelli A. 2006. Identification of endangered Mediterranean cyprinodontiform fish by means of DNA intersimple sequence repeats (ISSRs). *Biochemical Systematics and Ecology*, (34)8 : 626-634.
17. Nei, M. and W.H. Li 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci., USA, 76: 5269-5273.
18. Rashed, M. A., Y. M. Saad, A. A. EL - Seoudy and M. M. Ibrahim 2009. Gene flow in some *Oreochromis niloticus* populations based on SSR linked markers to MHC loci class I. J. Boil. Chem. Environmen. Sci., 4(1) 319 - 331.
19. Rashed, M. Abd - Elsalam, Y. M. Saad, M. M. Ibrahim and A. A. EL - Seoudy 2008. Genetic structure of Natural Egyptian *Oreochromis niloticus* evaluated from dominant DNA markers. Global Veterinaria, 2(2): 87 - 91.
20. Rashed, M.A., A. A. EL - Gamal, T. M. A. Tantawy and Y. M. Saad 1998. Detection of some *Oreochromis niloticus* lines by isozyme organ distribution and PCR - RAPD DNA markers.3rd Arab conference. Modern Biotech. & Areas of application in the Arab world, 14 - 17 December 1998, Cairo, Egypt, Pp. 127 - 146.
21. Rashed M. A., Y. M. Saad, A. H. Atta and N. E. Ahmed 2011. Reconstruction of phylogenetic relations among Four Tilapia species. World Applied Science Journal (14)3:456-462.
22. Reddy M., Sarlan and Siddiq E. 2002. Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica*, (128)1: 9-17.
23. Rohlf, F.J. 1997. Manual of the *NTSYSpc2.01b* software. Applied Biostatistics Inc.
24. Saad Y. M., Rashed, M.A., S. I. EL - Deep, A. A. EL - Gamal and M. Saiid 2002. Molecular Genetic markers and phylogenetic relations for some Tilapia species. J. of Union Of Arab Biol. Cairo. Vol. 18(A): Zoology, 27 - 44, Sep.
25. Saad, Y. M., A. A. Mansour and A. M. EL - Nagar 2009. Monitoring of genetic polymorphism in some tilapia species based on fin tissues isozyme distributions. World J. Zool., 4(1): 24 – 28.
26. Tong J., X. Yu and X. Liao 2005.Characterization of a highly conserved microsatellite marker with utility potentials in cyprinid fishes. J. Appl. Ichthyol., (21) 232–235.
27. Trewavas, E. 1983. Tilapiine fishes of the genera *Sarotherodon, Oreochromis* and *Danakilia*. British Museum (Natural History). London, Uk.583 p.
28. Yun-Guo, Song-Lin Chen, Jing Li and Ba-Fang Li 2006. Genetic diversity in three Japanese flounder (*Paralichthys olivaceus*) populations revealed by ISSR markers. Aquaculture (255), Issues 1-4, 565-572
29. Zietkiewicz, E.; A. Rafalski and D. Labuda 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics, 20: 176-183.

11/12/2012

Evaluation of hematological and biochemical effects of pefloxacin/ diclofenac interaction in goat

El-Ghoneimy[1] A. A. and Shaheen[2] H. M.

[1]Department of Pharmacology, Faculty of Vet. Med., Qena, South Valley University, Egypt.
[2]Department of Pharmacology, Faculty of Vet. Med., Damanhour University, Egypt.
dr_hazemshaheen3010@yahoo.com

Abstract: Co-administration of Nonsteroidal Anti-inflammatory Drugs (NSAIDs) and antimicrobials may occur frequently because of worldwide in medical as well as veterinary practices. Often it is either diminished therapeutic efficacy or increased toxicity of one or more of the administered drugs. In this study the effect of pefloxacin/ diclofenac interaction was investigated on twenty clinically healthy goats of balady breed with an average body weight (21-27 kg). Goats were injected i.m. with pefloxacin at dose rate of 5 mg/kg b.wt daily for 4 days consequently. Diclofenac sodium was injected i.m. at dose rate of 1 mg/kg b.wt daily for 4 days consequently to different groups of goats . Two blood samples were collected from each animal of all groups at 3rd day, then at 1st , 2nd , 3rd week post injection. The obtained results revealed that co-administration of diclofenac sodium and pefloxacin showed significant changes ($p < 0.05$) in total erythrothytic count (RBCs), haemoglobin (Hb) % and packed cell volume (PCV). no significant change in total leucocytic count . Drugs caused slight impairment of hepatic and renal functions of goats. The effect of both drugs was short-lived and most of the parameters went back to normal after 2 weeks post drug(s) administration.

[El-Ghoneimy A. A. and Shaheen H. M. **Evaluation of hematological and biochemical effects of pefloxacin/ diclofenac interaction in goat.** *Life Sci J* 2012;9(4):4847-4854] (ISSN:1097-8135). http://www.lifesciencesite.com. 729

Keywords: Hematological; Biochemical; Pefloxacin; Diclofenac; goat

1. Introduction

Inflammation and pain mostly occurs in many diseases of goats. To provide improvement in animals well being and outcome, the condition of inflammation and pain is treated /managed by a variety of pharmacological agents of which NSAID's and antimicrobials are one of the important and large group (Aydin *et al*., 2003).

NSAID's are used for the symptomatic treatment and management of inflammation, fever and or pain associated with disease or injury of domestic livestock (George, 2003). Diclofenac is used in a variety of painful conditions like osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, renal colic, acute muscle pain, in dentistry and preoperatively to reduce postoperative pain. Beside its therapeutic effects some significant adverse effects are also associated with diclofenac in different species (Katzung, 1998; Bhagat *et al.,* 2003 ; Ahmad, 2008).

Co-administration of several drugs often results in unpredictable therapeutic outcome. Often it is either diminished therapeutic efficacy or increased toxicity of one or more of the administered drugs. Various pharmacokinetic interactions between antimicrobials and NSAIDs have been described (Chambers and Jawetz, 1997; Brooks *et al.,* 1998; Harry *et al.,* 1998; Barbosa *et al.,* 2001; Ahmed *et al.,* 2005).

Pefloxacin, is a synthetic antibacterial agent of the flouroquinolone group developed exclusively for veterinary use. It is mainly indicated for gastrointestinal, urogenital, skin and respiratory tract infections caused by gram-positive and gram-negative bacteria as well as mycoplasma in various domestic animal species (Moutafchieva and Yarkov, 2006).

Because of the published information on the pharmacology of both drugs in the goat is very limited, however, there is a need to investigate in this species their pharmacodynamics (PD), when administered both singly and in combination These data are required for determining dosage schedules for clinical use.

This study has therefore been designed to investigate the safety or potential toxicity of pefloxacin / diclofenac sodium usage in goats on some haematological and biochemical parameters that indicating general health condition in goat specie. This study will help to develop an opinion regarding the concern raises for its clinical use in animals.

2.Materials and Methods

Experimental animals

A total of twenty healthy adult female native Baladi goats with an average body weight of 21.6 ± 2.24 kg (mean ± SE) were obtained from the farm at South Valley University. The animals were housed in

hygienic stables and fed an antibiotic-free diet for at least 30 days before the study. For each treatment period, the animals were observed daily for general health. Clinical observations were made prior to injection and at 2, 10 and 24 hrs post-injection. The animals were maintained under optimal nutritional conditions and fed barseem, a drug-free concentrate, and water *ad libitum*.

Drugs and treatment

Peflodad® **(pefloxacin 10%)** was obtained from Dar Aldawa Veterinary and Agriculture Industrial Co. Ltd. Jordan. Each ml contains 100 mg of pefloxacin base.

Dicloflame ® **(diclofenac sodium 2.5%)** was obtained from Unipharma Elobour City, Cairo, Egypt. Each ml contains 25 mg of diclofenac sodium.

The goats were divided into 4 equal groups (each of 5):

Group 1. Goats were kept without any medication as control group.

Group 2. Pefloxacin was injected intramuscularly at a dose of 5 mg kg^{-1} (recommended dose) daily for 4 days consequently ,pefloxacin treated group.

Group 3. Diclofenac sodium was injected intramuscularly at a dose of 1 mg kg^{-1} (recommended dose) daily for 4 days consequently ,diclofenac sodium treated group.

Group 4. Both pefloxacin and diclofenac sodium were injected intramuscularly at the same dose rate for 4 days consequently, pefloxacin/diclofenac sodium treated group. The injection had occurred at two different sites.

Sample collection

Two blood samples were collected from each animal of all groups at 3rd day of injection then at 1st, 2nd, 3rd week post injection. The first blood sample was collected in test tubes containing EDTA for hematological studies. While the second blood samples were allowed to coagulate at 4 °C and were then centrifuged at 3000 × g for 15 min to separate the serum. The serum samples were frozen at −20°C.

Hematological Analysis

The hematological parameters, Red blood cells (RBCs) count, White blood cells (WBCs) counts, Hemoglobin (Hb%), Packed Cell Volume (PCV%) and Red blood cells indices including Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were estimated by using automatic cell counter (Exigo, Veterinary Hematology System, Boule Medical AB, Stockholm, Sweden.).

Biochemical Analysis

Biochemical parameters alanine transaminase (ALT) and aspartate transaminase (AST), total proteins, albumin, blood urea nitrogen, serum uric acid and serum creatinine . were measured. The Biochemical parameters were analyzed by commercially available kit methods. Globulins were estimated by electrophoretic analysis of serum protein.

Statistical analysis

The descriptive data are presented as the means ± SE. The statistical differences were calculated on the basis of two way test of ANOVA and $p < 0.05$ is considered as significant between the groups. The data were statistically analyzed by using (One way ANOVA test for variance analysis) (Student-Newman-Keuls) at $p < 0.05$ (Quinton *et al.,* 1995).

3. Results

All goats were clinically healthy throughout the experiment. None of the goats in all groups suffered from identifiable reactions following the administration of pefloxacin or diclofenac.

Hematological Analysis

Intramuscular administration of both diclofenac sodium at a dose rate of 1 mg kg^{-1} and pefloxacin at a dose of 5 mg kg^{-1} revealed significant effect on total erythrocytic count , haemoglobin % and packed cell volume (Table 1) .

A significant drop ($p < 0.05$) in total erythrocytic count at 3rd day post treatment. The total erythrocytic count was (8.05 ± 0.37) with respect to (9.41 ± 0.21 ; 9.57 ± 0.23 and 12.53 ± 0.56 in control goats respectively).

Hemoglobin % decreased significantly till the 1st week post drug administration. It was (8.04± 0.18) vs. (8.70 ± 0.11; 9.83 ± 0.10 and 10.20 ± 0.12 in control goats respectively).

Hemoglobin % decreased significantly till the 1st week post drug administration. It was (8.04± 0.18) vs. (8.70 ± 0.11; 9.83 ± 0.10 and 10.20 ± 0.12 in control goats respectively).

Packed cell volume (%) decreased significantly till the 1st week post drug administration. It was (26.67 ± 0.67) vs. (29.14 ± 0.50; 30.55 ± 0.88 and 34.67 ± 0.45 in control goats respectively).

By the 2nd week post drug administration, the total erythrocytic count, Hemoglobin and Packed cell volume values returned to normal levels in all groups.

No statistically relevant differences in total leucocytic count that remained within the same values between all groups (Table 3).

Biochemical Analysis

Intramuscular administration of both diclofenac sodium at a dose rate of 1 mg kg^{-1} and

pefloxacin at a dose of 5 mg kg^{-1} revealed significant effect on ALT, AST, serum total proteins, serum albumin , serum globulin, serum creatinine, serum uric acid and blood urea values.

A significant increase (p<0.05) in serum level of ALT was recorded at 72 hrs post treatment (Table 4).

AST increased significantly at 72 hrs and still examined by the end of the 1st week post treatment (36.0 ± 1.73) with respect to (31.0 ± 0.58; 25.0 ± 1.12 and 23.33 ± 0.88) in different groups (Table 4).

Total protein values was significantly dropped at 72 hrs post treatment and then gradually returned to base line values (Table 5).

Serum creatinine, uric acid and blood urea values increased significantly at 72 hrs post treatment. Both serum creatinine and blood urea were still examined by the end of the 1st week post treatment (Table 6).

Table 1. Effect of intramuscular injection of pefloxacin (5 mg kg^{-1}) or diclofenac sodium (1 mg kg^{-1}) and both drugs for 4 consecutive days on total erythrocytic count, hemoglobin concentration and Packed cell volume values of healthy goats (n=5).

Parameters / Groups	Erythrocytic count (10^6/mm)				Hemoglobin concentration (%)				Packed cell volume (%)			
	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week
Control	12.53 ± 0.56 a	11.97 ± 0.70 a	11.84 ± 0.31 a	11.80 ± 0.31 a	11.38 ± 0.09 a	10.20 ± 0.12 a	10.27 ±0.2 0 a	10.15 ± 0.19 a	34.22 ± 0.73 a	34.67 ± 0.45 a	33.15 ± 0.88 a	33.17 ± 1.20 a
Pefloxacin	9.57 ± 0.23 b	10.97 ± 0.75 ab	10.53 ± 0.82 a	10.75 ± 0.68 a	9.07 ± 0.20 b	9.83 ± 0.10 a	9.98 ± 0.17 a	9.96 ± 0.10 a	26.33 ± 0.53 b	30.55 ± 0.88 b	31.09 ± 0.70 a	31.80 ± 0.98 a
Diclofenac sod.	9.41 ± 0.21 b	9.83 ± 0.38 b	10.65 ±0.26a	10.65± 0.40 a	8.47 ± 0.13 b	8.70 ± 0.11b	9.95± 0.12a	9.93 ± 0.18 a	25.94± 0.33 b	29.14 ± 0.50 b	30.95 ± 0.57 a	31.67 ± 0.67 a
Pefloxacin + Diclofenac sod.	8.05 ± 0.37 c	9.42 ± 0.29 b	10.32± 0.29 a	10.47 ±0.33a	7.83 ± 0.08c	8.04± 0.18 c	9.76 ± 0.10a	9.76 ± 0.12 a	22.00 ± 0.58c	26.67 ± 0.67 c	30.67 ± 1.2 a	30.93 ± 0.68 a

Values are expressed as Mean ± SE.
The means which carry different letter in the same column were significantly different, (p < 0.05).

Table 2. Effect of intramuscular injection of pefloxacin (5 mg/kg^{-1}) or diclofenac sodium (1 mg/kg^{-1}) and both drugs for 4 consecutive days on mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) of healthy goats (n=5).

Parameters / Groups	Mean corpuscular volume (MCV)				Mean corpuscular hemoglobin (MCH)				Mean corpuscular hemoglobin concentration (MCHC)			
	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week
Control	27.31 ± 0.83 a	28.96 ± 1.98a	28.00± 0.12 a	28.11 ± 0.25a	9.08 ± 0.51 a	8.52 ± 0.69 a	8.67 ± 0.12 a	8.60 ± 0.48 a	32.18 ± 0.96 a	29.42 ± 0.98 a	30.98 ± 0.31 a	30.60 ± 1.77 a
Pefloxacin	27.51 ± 1.12 a	27.85 ± 1.24a	29.53± 1.57 a	29.58 ± 2.2 a	9.48± 0.23 a	8.99 ± 0.02 a	8.58 ± 0.63 a	9.27 ± 0.5 a	34.45 ± 0.31a	32.18± 0.83 a	32.10± 1.24 a	31.32 ± 0.65 a
Diclofenac sod.	27.57 ± 0.32 a	29.64 ± 0.65a	29.06 ± 0.28a	29.74 ± 0.5 a	9.00 ± 0.14 a	8.85 ± 0.26 a	9.11 ± 0.24 a	9.32 ± 0.23 a	32.65 ± 0.45 b	29.86 ± 0.23 a	32.15 ± 0.55 a	31.36 ± 0.49 a
Pefloxacin + Diclofenac sod.	27.33 ± 0.68 a	28.31 ± 0.23a	29.72 ± 0.35a	31.40 ± 0.62a	9. 72± 0.27 a	8.54 ± 0.12 a	9.46 ± 0.38 a	9.32 ± 0.34 a	35.59± 0.35 c	30.15 ± 0.25 a	31.82 ± 1.51 a	31.56 ± 0.7 a

Values are expressed as Mean ± SE.
The means which carry different letter in the same column were significantly different, (p < 0.05).

Table 3. Effect of intramuscular injection of pefloxacin (5 mg/kg^{-1}) or diclofenac sodium (1 mg/kg^{-1}) and both drugs for 4 consecutive days on total leucocytic count (cell/mm^3) of healthy goats (n=5).

Parameter / Groups	Total leucocytic count (cell/mm^3)			
	3rd day	1st Week	2nd Week	3rd Week
Control	7.83 ± 0.4 a	7.82 ± 0.27 a	7.63 ± 0.13 a	7.18 ± 0.42 a
Pefloxacin	7.54 ± 0.27 a	7.48 ± 0.12 a	7.25 ± 0.2 a	7.53 ± 0.23 a
Diclofenac sod.	7.63 ± 0.04 a	7.60 ± 0.14 a	7.57 ± 0.12 a	7.34 ± 0.01 a
Pefloxacin + Diclofenac sod.	7.49 ± 0.35 a	7.95 ± 0.34 a	7.80 ± 0.35 a	7.67 ± 0.32 a

Values are expressed as Mean ± SE.
The means which carry different letter in the same column were significantly different, (p < 0.05).

Table 4. Effect of intramuscular injection of pefloxacin (5 mg/kg^{-1}) or diclofenac sodium (1 mg/kg^{-1}) and both drugs for 4 consecutive days on serum alanine aminotransferase enzyme (ALT, IU/L) and serum asparatate aminotransferase enzyme (AST, IU/L) of healthy (n=5).

Parameters / Groups	Serum Alanine Aminotransferase (ALT)				Serum Asparatate Aminotransferase (AST)			
	3rd day	1st week	2nd week	3rd week	3rd day	1st week	2nd week	3rd week
Control	24.27 ± 0.87 ^{d}	24.78 ± 0.94 ^{a}	24.70 ± 0.92 ^{a}	24.01 ± 0.75 ^{a}	21.67 ± 0.80 ^{d}	23.33 ± 0.88 ^{c}	24.67 ± 1.11^{a}	20.0 ± 1.03 ^{a}
Pefloxacin	45.38 ± 0.65 ^{c}	25.54 ± 0.74 ^{a}	25.87 ± 0.76 ^{a}	24.09 ± 0.38 ^{a}	39.33 ± 1.19 ^{c}	25.0 ± 1.12 ^{c}	23.0 ± 1.03 ^{a}	24.33 ± 1.17 ^{a}
Diclofenac sod.	64.01 ± 0.77 ^{b}	27.40 ± 1.05 ^{a}	26.31 ± 0.81 ^{a}	25.13 ± 0.91 ^{a}	53.0 ± 0.58 ^{b}	31.0 ± 0.58 ^{b}	24.0 ± 1.10 ^{a}	25.0 ± 1.60 ^{a}
Pefloxacin + Diclofenac sod.	76.46 ± 0.93 ^{a}	27.90 ± 1.06 ^{a}	26.83 ± 1.04 ^{a}	25.48 ± 0.87 ^{a}	89.0 ± 1.31 ^{a}	36.0 ± 1.73 ^{a}	25.0 ± 1.06 ^{a}	28.0 ± 1.50 ^{a}

Values are expressed as Mean ± SE.

The means which carry different letter in the same column were significantly different, ($p < 0.05$).

Table 5. Effect of intramuscular injection of pefloxacin (5 mg/kg^{-1}) or diclofenac sodium (1 mg/kg^{-1}) and both drugs for 4 consecutive days on serum total proteins (g/dl), serum albumin (g/dl) and serum globulin (g/dl) levels of healthy goats (n=5).

Parameters / Groups	Serum total protein (g/dl)				Serum albumin (g/dl)				Serum globulin (g/dl)			
	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week
Control	6.94 ± 0.12^{a}	6.52 ± 0.21 ^{a}	6.79 ± 1.26 ^{a}	6.88 ± 0.15 ^{a}	4.92 ± 0.21 ^{a}	4.62 ± 0.26 ^{a}	4.56 ± 0.20 ^{a}	4.81 ± 0.17 ^{a}	2.02 ± 0.10 ^{a}	1.90 ± 0.21 ^{a}	2.23 ± 0.14 ^{a}	2.07 ± 0.25 ^{a}
Pefloxacin	6.48 ± 0.41^{a}	6.15 ± 0.33 ^{a}	6.18 ± 1.0 6^{a}	6.70 ± 0.21 ^{a}	4.56 ± 0.16 ^{a}	4.20 ± 0.24 ^{ab}	4.36 ± 0.16 ^{a}	4.77 ± 0.16 ^{a}	1.92 ± 0.11 ^{a}	1.95 ± 0.14 ^{a}	1.82 ± 0.22 ^{a}	1.93 ± 0.15 ^{a}
Diclofenac sod.	4.89 ± 0.31 ^{b}	5.34 ± 0.19 ^{b}	6.06 ± 0.18 ^{a}	6.48 ± 0.17 ^{a}	3.66 ± 0.11 ^{b}	3.50 0.15 ^{bc}	4.24 ± 0.12 ^{a}	4.68 ± 0.18 ^{a}	1.23 ± 0.24 ^{b}	1.84 ± 0.28 ^{a}	1.82 ± 0.10 ^{a}	1.80 ± 0.14 ^{a}
Pefloxacin + Diclofenac sod.	4.26 ± 0.18 ^{b}	4.89 ± 0.31 ^{b}	5.68 ± 0.17 ^{a}	6.30 ± 0.23 ^{a}	3.11 ± 0.13 ^{c}	3.21 ± 0.21 ^{c}	3.92 ± 0.22 ^{a}	4.65 ± 0.26 ^{a}	1.15 ± 0.15 ^{b}	1.68 ± 0.12 ^{a}	1.76± 0.13 ^{a}	1.65 ± 0.12 ^{a}

Values are expressed as Mean ± SE.

The means which carry different letter in the same column were significantly different, ($p < 0.05$).

Table 6. Effect of intramuscular injection of pefloxacin (5 mg/kg^{-1}) or diclofenac sodium (1 mg/kg^{-1}) and both drugs for 4 consecutive days on serum urea (mg/dl), serum uric acid (mg/dl) and serum creatinine (mg/dl) levels of healthy (n=5).

Parameters / Groups	Serum urea (mg/dl)				Serum uric acid (mg/dl)				Serum creatinine (mg/dl)			
	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week	3rd day	1st Week	2nd Week	3rd Week
Control	14.36 ± 0.18 ^{d}	14.42 ± 0.22 ^{c}	15.05 ± 0.21 ^{a}	14.92 ± 0.15 ^{a}	0.87 ± 0.16 ^{b}	0.87 ± 0.21 ^{a}	0.80 ± 0.20 ^{a}	0.83 ± 0.40 ^{a}	0.90 ± 0.12 ^{c}	0.91 ± 0.11 ^{c}	0.91 ± 0.19 ^{a}	0.94 ± 0.14 ^{a}
Pefloxacin	17.87 ± 0.25 ^{c}	14.94 ± 0.13 ^{c}	15.23 ± 0.24 ^{a}	15.02 ± 0.12 ^{a}	1.69 ± 0.21 ^{a}	0.89 ± 0.11 ^{a}	0.85 ± 0.31 ^{a}	0.87 ± 0.55 ^{a}	1.43 ± 0.15 ^{b}	1.27 ± 0.10 ^{bc}	1.04 ± 0.22 ^{a}	1.01 ± 0.11 ^{a}
Diclofenac sod.	26.05 ± 0.22 ^{b}	16.63 ± 0.18 ^{b}	15.44 ± 0.36 ^{a}	15.13 ± 0.11 ^{a}	1.82 ± 0.22 ^{a}	1.03 ± 0.31 ^{a}	0.90± 0.25 ^{a}	0.89 ± 0.24 ^{a}	1.68 ± 0.11 ^{b}	1.52 ± 0.12 ^{ab}	1.08 ± 0.30 ^{a}	1.07 ± 0.17 ^{a}
Pefloxacin + Diclofenac sod.	31.09 ± 0.11 ^{a}	19.22 ± 0.16 ^{a}	15.97 ± 0.29 ^{a}	15.45 ± 0.19 ^{a}	1.98 ± 0.32 ^{a}	1.10 ± 0.20 ^{a}	0.98 ± 0.41 ^{a}	0.95 ± 0.34 ^{a}	2.38 ± 0.13 ^{a}	1.76 ± 0.22 ^{a}	1.10 ± 0.21 ^{a}	1.13 ± 0.30 ^{a}

Values are expressed as Mean ± SE.

The means which carry different letter in the same column were significantly different, ($p < 0.05$).

4.Discussion

Fluoroquinolones are increasingly employed in medicine to eradicate the susceptible bacterial infection. Co-administration of several drugs often may result in unpredictable therapeutic outcome. The use of non-steroidal anti-inflammatory drugs (NSAIDs) are frequently recommended with antibacterials for the treatment of various bacterial infections accompanied by fever and other inflammatory conditions in the men and animals. It is widely recognized that co-administration of two drugs may affect the absorption, distribution, biotransformation and/or excretion of both agents (Patel *et al.*, 2011).

Following concurrent intramuscular administration of diclofenac and enrofloxacin in sheep, there was a significant rise in the plasma concentration of diclofenac, the peak value being 7.74 µg ml^{-1} observed at 0.5 h. The plasma concentration of diclofenac was maintained above therapeutic plasma level(3.43 µg ml^{-1}) for 7 hrs after drug administration. However, these differences were not statistically significant, which suggests that diclofenac did not influence the kinetics of enrofloxacin and ciprofloxacin (Kumar *et al.,* 2003). Also, intramuscular administration of moxifloxacin did not produce significant influence over the elimination processes of intramuscularly

administered tolfenamic acid in the male and female rats (Patel *et al.*, 2011).

On contrary, enrofloxacin inhibits hepatic microsomal cytochrome P450 monooxygenases (Shlosberg *et al.,* 1997). There is a possibility that concurrent administration of enrofloxacin resulted in decreased oxidation of diclofenac due to which the plasma levels of diclofenac were maintained for a longer period. Moreover, fluoroquinolones have previously been reported to decrease the toxicity of acetaminophen by preventing its biotransformation by inhibiting cytochrome P450 isozymes (Nakashi and Okuno,1990).

So, the result of the study indicates that diclofenac when administered with pefloxacin in goats, causes significant effect on some hematological, liver and kidney function parameters could be associated with diclofenac effect.

Intramuscular administration of both diclofenac sodium at a dose rate of 1 mg kg^{-1} and pefloxacin at a dose of 5 mg kg^{-1} revealed significant effect on red cell related parameters. This could attributed to diclofenac induced immune hemolysis produce a broad spectrum of anti-diclofenac/RBCs antibodies. 4'-OH- diclofenac seems to represent the most immunogenic metabolite. Serum could contain a mixture of antibodies that recognize several and distinguishable epitopes. These epitopes consist of different drug metabolites and a target protein on the RBC surface. So, diclofenac forms neoantigens with RBCs that may stimulate the production of autoantibodies and drug-dependent antibodies (Salama *et al.,*1996 ; Sachs *et al.,*2004).

These findings could explain the significant decrease in globulins levels in both diclofenac and diclofenac/pefloxacin treated goats in comparison to the other two groups. On the other side, the level of leucocytic count remain unchanged, so, this may approve that that hemolytic anemia induced by diclofenac may be due to cellular and humoral immunity as well. According to the association of hemolytic anemia with duration of

treatment we found that hemolytic anemia was accompanied with diclofenac treatment and was drug dependent as upon discontinuation of treatment we noticed correction of

hemoglobin concentration.

Despite the fact that diclofenac itself was effective in impairing ATP synthesis by mitochondria, there was evidence that toxicity was also related to the drug metabolism and was reduced by the addition of cytochrome p-450 inhibitors to the culture medium (Bort *et al.,1999*). Moreover, the key role of mitochondrial dysfunction in the pathogenesis of diclofenac-induced hepatocyte injury, as a result of the decrease of ATP and MPT, has also been recently reported (Masubuchi *et al.*, 2002). Since MPT is considered a major common mechanism for drug-induced hepatocyte necrosis and apoptosis (Higuchi *et al.,* 2001), it is very likely that apoptosis is involved in the adverse effect of diclofenac.

The result of the presented study indicates that intramuscular administration of both diclofenac sodium at a dose rate of 1 mg kg^{-1} and pefloxacin at a dose of 5 mg kg^{-1} revealed significant effect on liver and kidney in goats.

To determine the effect of diclofenac sodium on liver function was done to evaluate the functional status of liver. The results of this study are in the line of early reported findings (Aydin *et al.,* 2003; Schwaiger *et al.,* 2004; Ahmad *et al.,* 2012**)**.

Significant changes occurred in serum level of ALT, AST, total proteins, albumin and globulins in goats after diclofenac administration. These parameters are functional indicators of liver. Functional and structural alteration in liver leads to increased level of these enzymes in circulation (O'Connor *et al.,* 2003). AST, and ALT are present in liver cells. These enzymes are intracellular and are being located in mitochondria or cytoplasm or both and when cell's function altered, damaged or destroyed, the enzyme escapes into the blood (O'Connor *et al.,* 2003).

Also, it has been reported that the damage in hepatocytes induced by diclofenac sodium and could be associated with an idiosyncratic reaction (Kertz-Rommel and Boelsteri, 1993).

The two serum proteins measured to assess liver function are albumin and globulin that could reflect the hepatic cell condition (Talwar and Srivastava, 2003). Albumin, produced only in the liver, is the major plasma protein that circulates in the blood stream.

Albumin is also very important in the transportation of many substances such as drugs, lipids, hormones, and toxins that are bound to albumin in the bloodstream. Once the drug or other substance reaches the liver, it is detached from the albumin and made less toxic by conversion to a water-soluble form that can be excreted, as albumin largely account on acidic drugs (e.g. nonsteroidal antinflammatory drugs) on their transport in plasma, so may interfere with drug kinetic (Baggot, 2001).

The reduction in the total protein could be attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of cytochrome P-450 enzymes leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides may leading to fatty liver (Suresh Kumar *et al.,* 2007).

The correction of liver biochemical results from the 2nd week post treatment could explain that

the toxic effects of diclofenac to liver cells could be reversible and dose-dependent (Kayaalp *et al.,1998*). Despite the fact that diclofenac itself was effective in impairing ATP synthesis by mitochondria, there was evidence that toxicity was also related to the drug metabolism and was reduced by the addition of cytochrome p-450 inhibitors to the culture medium (Aydin *et al.,* 2003), so combination of diclofenac with fluoroquinolones could be useful in correction of toxic effect due to diclofenac sodium in goat.

Another possible factor for such correction of liver biochemical results is reported by Javed *et al.* (2009). glomerular filtration rate being the highest in goats than other ruminants so, urinary excretion of ciprofloxacin revealed that the excretion rate of the drug was highest in goats, followed by sheep, buffaloes, and cows.

Significant increase occurred in the uric acid after diclofenac sodium administration alone and in combination with pefloxacin. These findings are in agreement with the finding of Reddy *et al.* (2006).

Data of total plasma protein in our study is significantly decreased that accompanied with diclofenac administration this could attributed to increase in liver activity to protein catabolism which indicated by high levels of serum uric levels in the presented study (Talwar and Srivastava, 2003). Diclofenac is more cytotoxic to drug metabolizing cells than to non-metabolizing cell lines (Bort *et al.,* 1999).

Uric acid is freely filtered by the glomerulus and is reabsorbed in the early renal proximal convoluted tubule via uric acid transporter followed by secretion and finally, postsecretory reabsorption .Hyperuricemia may occur either of an overall decrease in secretion or an increase in uric acid production, or both. (Enomoto *et al.,* 2002).

Hyperuricemia ultimately may resulted from renal disease, that includes the interstitial renal disease, as well as tubular injury (Mazzali *et al.,* 2002). The mitochondrion is clearly a target of diclofenac-induced nephrotoxicity as demonstrated by the oxidative stress and massive DNA fragmentation reported from studies with diclofenac *in vivo* . A decrease in ATP synthesis was demonstrated in isolated kidney mitochondria with glutamate/malate (Lin Eng *et al.,* 2008).

In our study the increased blood urea is observed with diclofenac. The results are in agreement with the findings reported by Aydin *et al.* (2003) and O´Connor *et al.* (2003) . It has been observed that diclofenac also influences the functions of kidney along with the liver. Urea is formed in the liver and represents the principal end product of protein catabolism and is excreted by the kidney. Nephrotoxic effect of diclofenac probably causes a decrease in the rate of excretion of urea nitrogen that may produce an increase in the concentration of urea (Aydin *et al.,* 2003).

As diclofenac, like all other NSAIDS, prevents the synthesis of prostaglandin by inhibiting the enzyme cyclooxygenase in the cells of the body (Laurence, 2006) .The kidney is extremely active in the synthesis and metabolism of prostaglandins. These compounds participate in several processes in renal physiology including auto-regulation of renal blood flow, glomerular filtration, modulation of rennin release, tubular ion transport and water metabolism. It is not surprising that diminished prostaglandin synthesis may be initiating events in the patho-physiological process of diclofenac sodium induced renal dysfunction (Yasmeent *et al.,* 2007 .

The increased level of serum Creatinine has been observed in the study. Same observation is also reported by Aydin *et al.* (2003) and Reddy *et al.* (2006).

As with urea, the rate of excretion is influenced by glomerular filtration rate (GFR), and any abnormalities that decrease GFR will result in an increase serum creatinine (Talwar and Srivastava,2003). Previous studies have shown that minor increase in serum creatinine can reflect a marked fall in glomerular filteration rate (Salomons *et al.,* 2003). Creatinine and blood urea is increased due to less glomerular filtration rate which is possibly impaired by diclofenac.

Goats treated with pefloxacin showed significant decrease in total erythrothytic (RBCs) count, haemoglobin (Hb) % and packed cell volume (PCV) at 3rd day and 1st week post treatment as compared to control group.

Dutta and Badhe (1999) mentioned that the rapid depression of bone marrow following use of ciprofloxacin suggests an idiosyncratic reaction. In one case, pancytopenia was observed only 2 days after use of ciprofloxacin, while in the other case, bleeding symptoms started 5 days after starting ciprofloxacin (even though the drug was stopped on the fourth day). Thus, it is possible that a single dose may cause bone marrow depression in rare instances.

Our results coincided with Amer and EL-Shaieb (1998) who recorded that intramuscular injection of rabbits with enrofloxacin at a dose level of 10 mg/kg b.wt. for five successive days induced a significant decrease in erythrocytic count, haemoglobin concentration, PCV, MCV, MCH and as compared to the control. Kar and Ghosh (2002) who stated that goats treated with consecutive graded dose levels of ciprofloxacin showed significant decrease in Hb (Haemoglobin) and PCV (Packed cell volume) as compared to the control. El-Ghoneimy *et al.*(2008) who reported that rats treated with

enrofloxacin for five days evoked a significant decrease in erythrocytic count and haemoglobin concentration (Hb%).

Goats treated with pefloxacin revealed a non significant change in total leucocytic count as compared to control group. These results are in agreement with El-Ghoneimy *et al.* (2008) who found that enrofloxacin treated rats for five successive days showed no significant changes in total leucocytic count.

Although fluoroquinolones are occasionally associated with mild, transient elevations in aminotransferase levels, serious acute liver injury is uncommon (Paterson *et al.,* 2012) .

These findings coincided with El-Ghoneimy *et al.* (2008) who found that enrofloxacin treated rats for five successive days provoked significant increase in serum ALT and AST enzymes level.

Animals treated with pefloxacin provoked a non significant change in serum total protein, albumin and globulin levels as compared with control group. These results are in accordance with Kar and Ghosh (2002) who recorded that treatment of rabbit with enrofloxacin daily for five successive days induced no significant change in plasma protein levels as compared with the control. El-Ghoneimy *et al.* (2008) who recorded that enrofloxacin treated rats for five successive days provoked non significant change in serum total protein, albumin and globulin levels as compared with control group. Shoorijeh *et al.* (2012) who reported that cats treated with enrofloxacin daily for 7 consecutive days showed no significance change in serum total protein levels as compared with the control groups.

Allergic nephropathy associated with quinolone antibiotics has been reported. The mechanism might be a hypersensitivity reaction. Norfloxacin has been incriminated as a cause of acute interstitial nephritis (AIN) as the histopathological finding. Ciprofloxacin-associated nephropathy has been reported in 28 cases, with AIN as the main histopathological finding. Clinicians should be aware of quinolone-associated AIN, which is a rare but potentially dangerous renal complication (Hadimeri *et al.,* 1997).

The increased blood urea, uric acid and creatinine levels post pefloxacin treatment was in harmony with the findings reported by Khodary and El-Sayed (1997) and El-Ghoneimy *et al.* (2008).

Finally, it could be concluded that combination of diclofenac with fluoroquinolones could be useful in correction of toxic effect due to diclofenac sodium in goat.

References

1. Ahmad, I., 2008. Diclofenac induced effects in goat. *Agris, FAO of United Nations*.
2. Ahmed, F.A., Mohan, P., Barua, C.C. and Dutta, D.J., 2005. Effect of intramuscular diclofenac sodium on pharmacokinetics of intravenous enrofloxacin in calves. *Indian J. Pharmacol.*, 37:189-190.
3. Ahmad, I., Qureshi, T. A., Khan, F. A., Mughal, S. A. K., Sadique, U., Shah, Z., Khan, S. and Hassan, Z. U. ,2012. Evaluation of biochemical effects of diclofenac sodium in goats. The *Journal of Animal and Plant Sciences*, 22 (2 Suppl.), 1-4.
4. Aydin, G. I., Alparslan, G., Umen, K.; Meral, N. C.; Ekr, U. E. K.; Nermin, K. and Kalp, G., 2003. Histopathologic Changes in Liver and Renal Tissues Induced by Different Doses of Diclofenac Sodium in Rats. *Turk. J. Vet. Anim. Sci.* ,27: 1131-1140.
5. Baggot. J. D.,2001. The Physiological Basis of Veterinary Clinical Pharmacology. Wiley-Blackwell, 92-135.
6. Barbosa, J., Barron, D., Cano, J., Jimenez-Lozano, E., Sanz-Nebot, V. and Toro, I. , 2001. Evaluation of electrophoretic method versus chromatographic, potentiometric and absorptiometric methodologies for determing pK(a) values of quinolones in hydroorganic mixtures. *J. Pharm. Biomed. Anal*., 2001;24:1087-1098.
7. Bhagat, H., Malhotra, K., Tyagi, C., Gangwar, N. and Pal, N., 2003. Evaluation of preoperative rectal diclofenac for perioperative analgesia in ENT surgery. *Indian J Anesthesia* 47: 463- 6.
8. Bort, R., Ponsoda, X., Jover, R., Gomez-Lechon, M.J., Castell, J.V., 1999. Diclofenac toxicity to hepatocytes: A role for drug metabolism in cell toxicity. *J. Pharmacol. Exp. Ther*., 288: 65-72.
9. Brooks, G.F., Butol, J.S. and Morse, S.A., 1998. In, Jawetz-Melnick and Adel bergs Medical *Microbiology*, 155.
10. Chambers, H.F. and Jawetz E. , 1997. Principles of antimicrobial drug action-Sulphonamides, trimethoprim and quinolones.: In: Basic and Clinical Pharmacology, Katzung BG (ed.) Stanford, Appleton and Lange, New York.: 119-120.
11. Dutta, T. K. and Badhe, B. A., 1999. Adverse drug reaction: Ciprofloxacin-induced bone marrow depression. *Postgrad. Med. J*.,75:571–573.
12. El-Ghoneimy, A.A., Shaheen, H.M., Mohi, M. M., El-Sheikh, W.M. and Ramadan, T.M. , 2008. Some adverse effects of interaction between Furosemide and enrofloxacin in albino rats. *Kafrelsheikh Vet. Med. J.,* Egypt. 6(2): 298-324.
13. Enomoto, A., Kimura, H., Chairoungdua, A., Shigeta, Y., Jutabha, P., Cha, SH., Hosoyamada, M., Takeda, M., Sekine, T., Igarashi, T., Matsuo, H., Kikuchi, Y., Oda, T., Ichida, K., Hosoya, T., Shimokata, K., Niwa, T., Kanai, Y. abd Endou, H., 2002. Molecular identification of a renal urate anion exchanger that regulates blood urate levels. *Nature*, 417 (6887): 447–52.
14. George, L. W., 2003**.** Pain Control in Food Animals**.** In: Recent Advances in Anesthetic Management of Large Domestic Animals, E.P. Steffey (Ed.) Publisher: International Veterinary Information Service (www.ivis.org), Ithaca, New York, USA.
15. Hadimeri, H., Almroth, G., Cederbrant, K., Eneström, S., Hultman, P., Lindell, A., 1997. Allergic nephropathy associated with norfloxacin and ciprofloxacin therapy.

Report of two cases and review of the literature. *Scand. J. Urol. Nephrol*.,31(5):481-5.

16. Harry, W., Lampiris, M.D. and Aniel, S.M., 1998. Clinical use of antimicrobial agents. *In: Basic and Clinical Pharmacol. 7th edn., Katzung, B. G. (ed) Stanford, Appleton and Lange, New York.:*812-826.
17. Higuchi, H., adachi, M., Miura, G.J. and Ishii, H., 2001.The mitochondrial permeability transition contributes to acute ethanol-induced apoptosis in rat hepatocytes. *Hepatology*, 34:320–4.
18. Javed, I., Iqbal, Z., Rahman, Z., Khan, M. Z., Muhammad, F., Khan, F. H., Sultan, J. I., 2009. Renal handling of ciprofloxacin in domestic ruminant species. Turk. J. Vet. Anim. Sci., 4 : 303-310.
19. Kar, B.K. and Ghosh, R.K., 2002. Haematological studies in goats treated with consecutive graded dose levels of ciprofloxacin. *Journal of Interacademicia*, 6(1): 73-76.
20. Katzung, B.G, 1998. *Basic and clinical pharmacology*, 7th ed., Connecticut; Appleton and Lange; pp 587-602.
21. Kayaalp, S.O., 1998. Rasyonel tedavi yönünden tıbbi farmakoloji. Medicine Pharmacology. 8. baskı. Ankara., Hacettepe Taş Kitapcılık, Ltd. Şti., 1032-1046.
22. Kertz-Rommel, D.A., Boelsteri, U.A. ,1993. Diclofenac covalent protein binding is dependent on acyl glucuronide formation and is inversely related to P 450-mediated acute cell injury in cultured rat hepatocytes. *Toxicol. Appl. Pharmacol*., 120: 155-161.
23. Khodary, R.M. And El-Sayed, E.M., 1997. Treatment of duckling salmonellosis by enrofloxacin. *Assuit Vet. Med. J.,* 36(72): 262-268.
24. Kumar, N. Singh, S.D. and Jayachandran, C. 2003. Pharmacokinetics of Enrofloxacin and Its Active Metabolite Ciprofloxacin and Its Interaction With Diclofenac After Intravenous Administration in Buffalo Calves. *The Veterinary Journal*, 165, 302–306.
25. Laurence, L. B., Bruce A. C. and Björn C. K., 2006. Goodman & Gilman's The Pharmacological Basis of Therapeutics, Eleventh Edition, The McGraw-Hill Companies New York.:673.
26. Lin Eng N.g., Halliwell, B. and Wong, K. P., 2008. Nephrotoxic cell death by diclofenac and meloxicam. Biochemical and Biophysical Research Communications, 369 , 873–877.
27. Masubuchi, Y., Ose, A. and Horie T., 2002.Diclofenac-induced inactivation of CYP3A4 and its stimulation by quinidine. *Drug Metab Dispos*., 10 :1143-8.
28. Mazzali, M., Kanellis, J. and Han, L., 2002. Hyperuricemia induces a primary renal arteriolopathy in rats by a blood pressure-independent mechanism. *Am. J. Physiol. Renal. Physiol.,* 282: 991-997.
29. Moutafchieva, R.and Yarkov, D., 2006. Pharmacokineticc of pefloxacin in birds. Trakia Journal of Sciences, 3, 28-33.
30. Nakashi, S. and Okuno, H., 1990. Comparison of inhibitory effects of new quinolones on drug metabolizing activity in the liver. *Japanese Journal of Pharmacology*, 53, 81–96.
31. O'Connor, N., Dargan, P.I. and Jones, A.L., 2003. Hepatocellular damage from non-steroidal
32.anti-inflammatory drugs. *Q. J. Med* ., 96:787–791.
33. Patel, S. D., Sadariya, K. A., . Gothi, A. K., Patel, U. D., Gohil, P. A., Jain, M. R., Bhavsar, S. K. and Thaker, A. M., 2011. Effect of Moxifloxacin administration on pharmacokinetics of tolfenamic acid in rats. *Braz. arch. biol. technol.*, 4 Curitiba. 739-744.
34. Paterson, J. M, Muhammad M. M., Manno, M.,Juurlink, D. N., 2012. Fluoroquinolone therapy and idiosyncratic acute liver. *CMAJ*, 184(14) : 1565- 1570.
35. Quinton, A., Lebedev, G., Youtz, P., Tuerke, T., Sinkler, R. and Kuo, J., 1995. *SigmaStat Version 2.0.* San Rafael, CA: Jandel Scientific.
36. Reddy, P. N. C., Anjaneyula, Y., Sivasankari, B. and Rao, A. K., 2006**.** Comparative toxicity studies in birds using nimesulide and diclofenac sodium Environ. Toxicol. Pharmacol., 22: 142-147.
37. Salomons, G.S., van Dooren, S.J., Verhoeven, N.M., Marsden, D., Schwartz, C., Cecil, K.M., DeGrauw, T.J. and Jakobs, C., 2003. X-linked creatine transporter defect: an overview. *J. Inherit. Metab. Dis*., 26:309–318.
38. Sachs, U.J., Santoso, S., Röder, L., Smart, E., Bein, G. and Kroll, H., 2004.Diclofenac-induced antibodies against red blood cells are heterogeneous and recognize different epitopes. *Transfusion*. 8 :1226-30.
39. Salama, A., Kroll, H., Wittmann, G. and Mueller-Eckhardt, C., 1996. Diclofenac-induced immune haemolytic anaemia: simultaneous occurrence of red blood cell autoantibodies and drug-dependent antibodies. *Br. J. Haematol.,* 4 , 640-4.
40. Shoorijeh S.J., Tamadon, A., Vahedi, M. and Behzadi, M.A., 2012. Hematological and biochemical alterations due to over dosage of enrofloxacin in cats. Pak. Vet. J., 32(1): 73-76.
41. Schwaiger, J., Ferling, H., Mallow, U. , Wintermayr, H. and Negele, R. D., 2004. Toxic effects of the non-steroidal anti-inflammatory drug diclofenac. Part I: histopathological alterations and bioaccumulation in rainbow trout. *Aquat. Toxicol*., 68,141-150.
42. Shlosberg, A., Ershov, E., Bellaiche, M., Hanji, V., Weisman, Y. and Soback, S., 1997. The inhibitory effects of the fluoroquinolone antimicrobials norfloxacin and enrofloxacin on hepatic microsomal cytochrome P-450 monooxygenases in broiler chickens. *Drug Metabolism Drug Interaction*, 2, 109–122.
43. Suresh Kumar, S.V., Sujatha, C., Syamala, J., Nagasudha, B. and Mishra, S.H., 2007. Hepatoprotective activity of extracts from *Pergularia daemia* Forsk against Carbon tetrachloride induced toxicity in rats. *Phcog. Mag*., 3,11.
44. Talwar, G.P., and Srivastava. L.M. , 2003. Textbook of Biochemistry and Human Biology. 3rd Ed, Prentice Hall of India Pvt.Ltd., 441-458.
45. Yasmeent, T., Qureshi, G. S. and Perveen, S., 2007. Adverse effects of Diclofenac sodium on renal parenchyma of adult albino rats. *Journal of Pak. Medical Association*, 7,349-51.

12/2/2012

A Study on the Price Transmission in Sharp-headed and White Shrimp Markets in Iran

Faramarz Ghalambaz[1],Reza Moghaddasi[1], Farshid Ghalambaz[1], Farhad Ghalambaz[2]

[1]Department of Agricultural Economics, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2] Department of Economics, Science and Research Branch, Islamic Azad University, Khozestan, Iran,
Corresponding Author : f.ghalambaz@ srbiau.ac.ir

Abstract: Aquatics are considered a suitable food source for providing consumable protein for people, and development of aquaculture in Iran is of special importance. Therefore, considering the importance of Price transmission in various levels of the market, and the relation between price transmission from farm to retail level with efficiency of the marketing system, the method of price transmission from wholesale to retail is examined regarding sharp-headed and white shrimp. For this purpose, wholesale and retail prices of two products during 2002 to 2011 were used in form of monthly data. Granger's causality test was used to examine the relation of causality between two levels of wholesale and retail prices. Test results indicate a one-way causality relation from the level of wholesale prices to retail prices. In addition, the most important findings of the research support the fact that price fluctuations on the wholesale level are asymmetrically transmissionred to the retail level. In other words, sensitivity of retail prices compared to the increase and decrease of wholesale prices are not equal.
[Faramarz Ghalambaz, Reza Moghaddasi, Farshid Ghalambaz, Farhad Ghalambaz. **A Study on the Price Transmission in Sharp-headed and White Shrimp Markets in Iran.** *Life Sci J* 2012;9(4):4855-4860] (ISSN:1097-8135). http://www.lifesciencesite.com.730

Keywords: Price Transmission, Causality Test, Price Transmission Elasticity, Houck's Model

1-Introduction

Providing food safety is one of the most important factors in creating a progressing society. Based on nutrition culture, some products withhold special positions. For example, the man main food of Asian countries, which is seen on most of their tables, consists of agricultural products and seafood. On the other hand, deterioration of agricultural products and aquaculture has a wide range of discussion, and warehousing of these types of products is very costly, and in undesirable conditions, will result in their food quality degradation.

Regarding shrimp products in Iran, we have witnessed price fluctuations, mainly because of seasonal price fluctuations of this product and production fluctuation due to bad weather and atmospheric conditions, and the Asian continent and the majority of producing countries like Iran are not an exception.

One of the other reasons affecting agricultural product prices is governmental intervention. Most of these interventions are through interference in agricultural product markets, sometimes in form of determining a bottom price to support of producers, and other times via providing different savings to producers with the aim of price cuts in favor of the consumer.

One of the major problems most markets are faced with is marketing margin, which has added importance in agricultural and fisheries products. Considering that production in the fisheries and agricultural field accounts for many risks, and has more production risks compared to industrial products, therefore the producer is under much more physical and psychological pressure. Therefore, marketing margin is of great importance in agricultural products.

Definition of Marketing Margin: Price difference between producer and consumer price is called Marketing Margin, which is mainly due to marketing services, which include product type, consumer characteristics, market structure, transportation system, packaging industry etc.

If increase or decrease of producer price does not affect the price the consumer pays, marketing margin will become more important, and this type of price transfer is called asymmetric transmission.

In Hans's point of view, there are two types of asymmetric transmission:

- Short-term: the immediate effect of increase or decrease in produce price does not affect retail price, but the long-term effect is equal.
- Long-term: increase in producer price has a different effect in the short-term compared to price reduction in the long-term. Long-term asymmetry means that intermediaries are continuously increasing their margin, while short-term asymmetry is considered a temporary effect on marketing margin. In order to find out the manner of price fluctuation transfer two economic models namely Hook and Error Correction are utilized. Hook's model is used if data are

non- stationary or are not in relation with each other on the long-term, and if data are nonstationary and are in relation with each other on the long-term, then the Error Correction model is used. Moreover, Convergence test is utilized to examine the long-term relations.

On-farm market includes:

- Seller: Farmer
- Buyer: wholesaler, retailer, and families

Intermediaries are persons familiar with market conditions and attempt to buy products from wholesalers and retailers.

Asymmetric transfer of prices is of great importance, because in addition to showing a great lack of economic theories, it can provide concepts and applications for economy policy makers.

Types of Price Transfers:

- Horizontal: price transfer of a product from one country's (region) market to another country's (region) market.
- Vertical: price transfer of a product in production levels is wholesaling and retailing inside the country.

A number of studies have been carried out in this regard. Among them are:

Kinokan and Focker (1987): the method of price transfer from the farm to retailers for four dairy products butter, cheese, raw milk, and ice cream was examined in the USA. Results showed that increase in farm prices compared to reduction of farm prices transfer in a faster and more complete manner to retail level, and price transfer has been done in an asymmetric way.

Agoyar and Kerno (1997) showed that in dairy processing industries of Brazil, increase on farm prices are transferred faster to retail.

Han (1990) concluded that beef and pork price transfer are asymmetric.

Keps and Sherol (2005) carried out the price symmetry test in four US states for raw milk and milk with 2% fat. Their results indicated asymmetric price transfers.

Ward (1982) regarding price transfer of fresh vegetables concluded that reduction of on-farm prices transfers in a faster and more complete manner.

Hosseini and Nikookar (2006) attempted to study the method of price transfer from producer to consumer for poultry in Iran during 1998 to 2002. Their results indicated that any increase in poultry producer prices transfers completely to retail, while this transfer is not complete.

Ghahremanzadeh and Falsafian (2005) by carrying out a study on the method of price transfer in Iran's meat market during 1991 to 2001, concluded that increase in producer price which results in reduction of marketing margin, transfers even faster to retail prices.

Moghadassi and Morab (2007) commenced a study on the method of price transfer from farm to retail in the tomato and potato product market. Results of their study indicates an asymmetric price transfer, and producer price increase is more complete, but is transferred to retail in a slower pace compared to price decrease.

Moghadassi and Ardakani (2007) carried out a study in examining the method of price transfer in the egg and poultry market of Iran. Their results validated the fact that price fluctuations in the producer level are symmetrically transferred to the retail level.

Moghaddasi and Fazeli (2007) studied garden product market price (case study on date sand pistachio nuts).

Hosseini and Doorandish (2006) examined the price transfer model of Iran's pistachio in the world market.

Hosseini and Saraeishad (2009) studied the method of price transfer in the trout fish culture in the Fars province. Their results validated the fact that price fluctuations on the wholesale level are transferred asymmetrically to the retail level.

2-Material and Methods

In this article, shrimp retail prices (Pr) and wholesale prices (Pf) were used in a monthly 10-year period from 2002 to 2011. Data were obtained from

Iran's Fisheries Organization. In order to eliminate inflation, Consumer Price Index (CPI) was employed.

In this research, price transmission, transmission prices elasticity, and causality relation between prices in two levels of wholesale and retail were examined and analyzed for two products, sharp-headed shrimp and white shrimp.

In later studies various methods have been employed in order to examine the method of price transmission in different levels of the market including Houck's method, Angel-Granger's convergence test, and error correction model.

Because data from the time series have been utilized, firstly, variable stationarity was examined using unit root test, Dickey- Fuller's statistic, Phillips-Perron and KPSS, and if the time series variables are stationary, Houck's model will be used. However, if variables are non-stationary, firstly, the long-term relationships of variables in different levels of the market are examined using Johansen's test, and if cointegrated, the error correction model will be used for analyzing price transmission model.

In order to study the method of price transmission, normally a quantity namely

transmission elasticity is used. As mentioned in the introduction, price transmission elasticity shows the percentage of retail price transmission for one percent of wholesale price change. In 1975, Gardner presented various equations for calculating this elasticity. However, we know that sources of price transmission are different. If product offer is the reason for price change, price transmission elasticity is lower than one, and if demand is the reason, provided that the agricultural product offer is more elastic compared to marketing savings offers, price transmission elasticity will be near or more than one. However, empirical studies have indicated that higher marketing services elasticity is due to agricultural products offer elasticity.

The following method are used in order to examine the method of price transmission in a market:

- Houck's model
- Error Correction Method (ECM)

In this article, monthly price time series data have been used. Therefore, some important tests must be carried out.

Granger's causality test is used in order to prices effective from each other. Dickey Fuller, Phillips-Perron, and KPSS tests are employed for examining stationarity.

A random process is stationary when the average and variance are constant in time and the covariance value between to time periods only depends on the distance or gap between the two periods, and does not relate to the actual time of real calculation of the variance.

If the absolute magnitude of the calculated statistic in Dickey Fuller and Phillips-Perron's tests is more than the absolute magnitude of critical values, then the hypothesis related to stationarity of the time series is not rejected. Otherwise, data are stationary. If data are stationary, a false regression will be produced, because both dependent and independent time series variables are greatly inclined to time, and therefore, the high value of observed R^2 is due to presence of a time variable, and not an outcome of actual relation between variables. Therefore, data must be stationary.

Now, if the time series is stationary, we use Houck's model:

$$Pr_t - Pr_0 = a_0 t + a_1 \sum_{i=0}^{M_1} \Delta Pf_{t-i}^{+} + a_2 \sum_{i=0}^{M_2} \Delta Pf_{t-i}^{-} + e_1$$

where Pr is logarithm of retail price, Pf is logarithm of price on the farm, ΔPf^{+} is price increase on the farm, ΔPf^{-} is price reduction on the farm, and M1 and M2 are gap lengths.

The variable coefficient equality hypothesis test is used to examine the symmetric or asymmetric nature of price positive and negative transmission shocks between the two levels of the market. $a1$ and $a2$ respectively, are coefficients of effect of increase and decrease of on-farm prices on wholesale prices.

This equation is estimated using the ordinary least squares method, and length of lag can be obtained using the Akaike -Schwartz or adjusted R^2 tests .

The null hypothesis can be defined as below:

$$H_0: \sum_{i=0}^{M_1} a_{1i} + \sum_{i=0}^{M_2} a_{2i}$$

The above equation is easily calculated using OLS.

If $a1$ and $a2$ are equal and positive, price transfer is symmetric, otherwise it is asymmetrical.

Wald's test is employed in order to test rejection or acceptance of the null hypothesis. This test is based on the F and X^2 statistics. Because all assumptions are linear, therefore in order to reject or accept each assumption, the F and X^2 statistics are compared with their critical values, and significance is specified in this regard.

Now, if Dickey Fuller's test results imply non-stationarity, the method of price transmission will change. Here we face two different conditions:

1. If data are cointegrated, meaning that they are in relation with each other on the long-term, the ECM model will be used. Johansen's test will be employed for data cointegration.
2. If data are not in relation to each other on the long-term, Houck's model will suffice.

Granger and Lee (1989) introduced the Error Correction Model (ECM) as follows:

$$\Delta P_{rt} = B_0 + B_1 \Delta P_{ft} + B_2^{+} ECT_{t-1}^{+} + B_2^{-} ECT_{t-1}^{-} + \sum_{i=1}^{P_1} B_{3i} \Delta P_{rt-i} + \sum_{i=1}^{P_2} B_{4i} \Delta P_{ft-i} + v_i$$

$$ECT_{t-1} = P_{rt-1} - a_0 - a_1 P_{ft-1} :$$

Resulting correction component from convergence regression between P_{rt} and P_{ft}:

In the above regression, retail price change to wholesale price change in the t time, and wholesale prices of the previous period were examined.

B2- and B2+ coefficients show the amount of retail price adjustments compared to positive and negative marketing margin shocks, respectively. The null hypothesis is defined as below:

$$H_0: B_2^{+} = B_2^{-}$$

Acceptance of the null hypothesis indicates symmetry in price transmission, and declining it represents asymmetry in price transmission. In this article, we attempt to examine causality between retail and wholesale markets, which finally, shows the effect of both these markets in different levels. In other words, through the causality test, we can

recognize which market is determinant and effective on price and price change in other markets.

In this article, Granger's causality test is used as follows:

$$Pw_t = \sum \propto_i P_{w_{t-i}} + \sum \beta_j Pr_{t-j} + U_{1t} \quad (1)$$

$$Pr_t = \sum \lambda_i P_{r_{t-i}} + \sum \delta_j Pw_{t-j} + U_{2t} \quad (2)$$

With the assumption of non-correlation of disorder components, the following four conditions are extractable:

1. If the sum of coefficients with the Pr interval in equation (1) are non-null statistically ($\sum \beta_j \neq 0$), and sum of coefficients with Pw interval in relation (2) are statistically zero ($\sum \delta_j \neq 0$), causality is one-way, from Pr to Pw.
2. If statistically $\sum \beta_j = 0$ and $\sum \delta_j \neq 0$, then causality is one-way from Pw to Pr, meaning that the wholesale market is the reason behind price changes in the retail market.
3. If the sum of Pw and Pr in both is statistically significant and noon-zero in both regressions, there is a two-way causality, and both markets influence each other.
4. If coefficients of Pw and Pr are not statistically significant in both regressions, then both markets are not in relation and are considered independent.

The optimal interval between equations (1) and (2) of the causality test must be determined for each variable. For the coefficients test, in each assumption of the causality test, parent coefficients test will be used.

3-Results and Discussion

Since data are time series, therefore firstly in order to examine stationarity of variables, the unit root test is used. By obtaining values for Dickey Fuller and Phillips-Perron tests, which in terms of absolute magnitude are larger that critical price values for both products in both levels of the market, therefore all variables are stagnant. Optimal interval for each variable is determined based on Akaike information criterion. Test results are given in table (1).

In order to examine the relation, Granger's causality test was employed. At first, the optimal interval for each variable was determined in each equation based on the least Akaike information criterion. For this, the price variable of each product in each level was regressed separately on its interval values, and optimal interval was determined in each equation for that variable. Then, the equation was regressed on the other variable in different intervals, and based on the least Akaike information criterion, the optimal interval for the other variable was determined. After determining variable optimal intervals, the following equations were appraised, and each of the assumptions of the causality test was examined based on the parent test. The results of this evaluation are given in table (2) to (4).

$$Pw_t = \sum \propto_i P_{w_{t-i}} + \sum \beta_j Pr_{t-j} + U_{1t}$$

$$Pr_t = \sum \lambda_i P_{r_{t-i}} + \sum \delta_j Pw_{t-j} + U_{2t}$$

Tables (2) and (3) present the causality test results between the two wholesale and retail of sharp-headed and white shrimp. It is evident that causality is rejected from retail to wholesale, and therefore, wholesale prices are not influenced by prices in the retail level. Based on this fact, for sharp-headed and white shrimp, there is a causal relation from wholesale to retail, and sharp-headed and white shrimp at the retail level, are influenced by wholesale market prices.

Table (3) presents results of the causality test between the two wholesale and retail levels of white shrimp. Based on the test results, null hypothesis is accepted in the first equation, therefore causality from retail to wholesale is rejected, and therefore prices in the wholesale level are not influenced by prices in the retail level.

Considering that all variables of the model are stagnant, therefore in order to examine the symmetry of price transfer in both levels of wholesale and retail, Hook's model was employed. For this, firstly, optimal interval of variables was determined in the model, and then the models were assessed. Assessment results are given in table (5). As observed in this table, for shrimp, price reduction or negative shocks have greater effect on retail prices, in a way that for sharp-headed shrimp, positive price shock in direction of wholesale in the same period, is transferred to the retail level with a coefficient of 0.4155, while price reduction influences the retail level with a coefficient of 0.4766. Hence, asymmetry that was defined in the article introduction is the outcome of this market, and positive and negative shocks from wholesale to retail are asymmetric. This result is validated using Wald's test. According to table (4), the zero-assumption on the basis of equal sum of price increase coefficients in various intervals, with the sum price reduction coefficients for both products, considering significance of the F statistic, is rejected. Therefore, price transfer in the shrimp market is asymmetric.

4-Conclusion

One of the problems of agricultural markets is lack of suitable tools for economy management

decisions. The current study with the aim of analyzing the price model and efficiency of the shrimp market, attempt to examine price transfer, marketing margin, and causality relations between different markets. Result of Engel-Granger's causality test showed that a causal relation exists from wholesale to the retail level.

Considering stationarity of variables, Hook's model was employed in order to examine the symmetry of price transfer between different levels of the market. Results showed that for these two markets, sum of price reduction coefficients or negative price shocks from wholesale to retail level had a faster effect to those of positive price shocks. Moreover, considering that variables are entered in the equation in logarithmic form, estimated coefficients indicated elasticity.

Table (1): Unit Root Test for Determining Stationarity of Shrimp (Sharp-headed and White) Price Variables,

Test		ADF			PP			KPSS	
Variable name		ADF Statistic	Critical values	p-value	PP statistic	Critical values	p-value	KPSS statistic	Critical values
Sharp-headed Shrimp	Wholesale	-3.950	-3.448	0.129	-4.078	-3.448	0.008	0.068	0.146
	Retail	-3.664	-3.448	0.000	-5.644	-3.448	0.000	0.079	0.154
White shrimp	Wholesale	-10.037	-3.448	0.000	-10.013	-3.448	0.000	0.062	0.146
	Retail	-10.432	-3.448	0.000	-11.842	-3.448	0.000	-0.104	0.146

Source: Study findings

Table (2): Causality Test Between Wholesale and Retail Levels of Sharp-Headed Shrimp

Causality relation	Rejection or acceptance of null- hypothesis	Parent test result	null- hypothesis	Model variables
Causality from retail to wholesale is rejected	H_0 accepted	F=0.8165 Probability = 0.4455	$\sum B_j = 0$	Pw, Pr (2, 2) Pr→*Pw*
Causality from wholesale to retail is accepted	H_0 rejected	F=2.6064 Probabaility=0.0009	$\sum \delta_j = 0$	Pr, Pw (2, 2) Pw→*Pr*

The numbers in parentheses are the optimal interval of each variable in the model.

Table (3): Causality Test between Wholesale and Retail Levels of White Shrimp

Causality relation	Rejection or acceptance of null- hypothesis	Parent test result	null- hypothesis	Model variables
Causality from wholesale to retail is accepted	H_0 rejected	F=4.0236 Probability = 0.0209	$\sum B_j = 0$	Pw, Pr (2, 2) *Pw* → Pr
Causality from retail to wholesale is rejected	H_0 accepted	F=0.8700 Probabaility=0.4221	$\sum \delta_j = 0$	Pr, Pw (2, 2) Pr → *Pw*

The numbers in parentheses are the optimal interval of each variable in the model.
Source: Research findings

Table (4): Hook's Model and Wald's Test Assessment Results for Symmetry of Various Types of Shrimp Price Transfer

Variable	Price change coefficient		Wald's test result	null- hypothesis acceptance or rejection	Price transfer symmetry
	Increase	Reduction			
Sharp-headed shrimp price	0.4155	0.4766	F=27.9205 Probability = 0.000	Rejected	Asymmetric
White Shrimp	0.6125	0.6792	F=3.8295 Probability = 0.0528	Rejected	Symmetric

Source: Research Findings

References

1-Fazeli, F., and Moghaddasifar, R. 2007. "Studying garden products price transmission: Case study on Dates and Pistachio". Proceedings from the 6th agricultural economy conference, Mashhad, Iran, 2006.

2-Goodwin, B. K., and Harper, D.C. 2000. "Price transmission, Threshold behavior, and Asymmetric adjustment in the U.S. pork sector". Journal of Agricultural and Applied Economics, 32,3, 2000.

3-Guillen, J., and Franquesa, R. 2007. "Analysis of the price transmission along the Spanish market chain for different seafood products". www.eafefish.eu

4-Hansen, B. 1994. "Determinants of the farm-retail milk price spread". Agricultural Information Bulletin, NP:693.

5-Hildreth, C., and Jarrett, F.G. 1995. "A statistical study of livestock production and marketing". Cowles Commission Monograph, No. 5 , New York.

6-Houck, J.P. (1997). "An approach to specifying and estimating Non-reversible function". American Journal of Agricultural Economics, 21, 30-59.

7-Hosseini, S, and Doorandish, A. 2006. "Iran's Pistachio price transmission model in the global market". Iran Agricultural Sciences Seasonal Journal, pp. 2-37.

8-Hosseini, S., and Nikookar, A. 2006. "Asymmetric price transmission and it's effect on marketing margin in Iran's meat and poultry market". Iran Agricultural Sciences Seasonal Journal, pp. 2-37.

9-Hosseini, S., and Saraei, Sh. 2009. "Price transmission in the Fars province Trout market". Journal of Agricultural Economics Researches,4(3)

10-Moghadassi,R.,and Ardakani,Z. 2007. "Studying the method of price transmission in Iran's egg and meat market". 6th Iranian Agricultural Economists Conference,Mashhad

11-Morab, A., and Moghaddasi, R. 2007. "Studying the method of price transmission from farm to agricultural products retailers: Case study on potatoes and tomatoes". 6th Iranian Agricultural Economists Conference, Mashhad.

12-Kinnucan H.W. and Forker O.D. 1987. Asymmetry in Farm-Retail Price Transmission for Major Dairyproducts. Amer. J. Agr. Econ. Vol. 69(2). pp. 285-292.

13-Aguiar D.R.D. and Connor J. M. 1997. The Effect of Changes in Regularory and Trade Policies on the Structure, Condoct and Performance of the Brazilian Dairy Processing Industry. Staff Paper No.97-15, Department of Agricultural Economic, Purdue University, West Lafayette. IN.

14-Capps J. O. and Sherwell P. 2005. Spatial Asymmetry in Farm-Retail Price Transmission Associated with Fluid Milk Products Selected Paper prepared for presentation at the American Agricultural Economics Association Annual Meeting, Providence, Rhode Island, July 24-27, 2005.

15-Von Cramon-Taubadel, S. (1998). Estimating asymmetric price transmission with the error correction representation: An application to the German pork market. European Review of Agricultural Economics, 25: 1-18.

16-Ward R.W. 1982. Asymmrtry in Retail, Wholesale and Shipping Point Pricing for Fresh Vegetables. Amer. J. Agr. Econ. Vol. 62. pp. 205-212.

12/12/2012

Effects of Environmental, Cultural, and Socioeconomic Factors on Saudi Infertile Couple in Riyadh City

Tahani Bin Aoun[1] and Salma Moawed[2]

[1, 2] Maternal & Child Health Nursing, Collage of Nursing, King Saud University, Riyadh, KSA
tbinaoun@KSU.EDU.SA

Abstract: Infertility is now recognized as a public health issue in the kingdom of Saudi Arabia, the prevalence rate of infertility is estimated to be more than 2.2% of the population, affecting about 30,000 couples. Infertility is affected by many different socioeconomic, cultural, and environmental factors, the latter playing a major role in infertility. The most cost effective of solving the infertility problem is prevention and education. The maternity nurse working in maternity filed or other agencies should be sensitive to the previous issue and share in health education program, through planning and implementation. The objective of the study were to assess of environmental, cultural, and socioeconomic factors through to affect human fertility among couples attending *in-vitro* fertilization clinics in three hospitals in Riyadh city (King Saud Medical Complex, King Fahad Medical City, and a private hospital). The study population consisted of Saudi couples coming for IVF process, all the patients participated voluntarily and shared in this study after signing a consent agreement .The instrument was adapted by the researchers of the Sher institution for reproductive medicine in Las Vegas, Nevada, United States of America in June 2006 and modified accordingly to the situation. A pilot Study was conducted to evaluate the tool. The data was collected over a period of nearly 3 months; starting from February 1st till the end of April 2010. It was carried out five days a week. Each couple was interviewed by subjects were assured that all the information gathered will be kept confidential. The age of the women on the study was between 26 and 30 years, the mean years of marriage was 4-7 years and most of them were housewives.51.5% of the female study subjects suffered with dysmenorrhia while 37.7% had pain during sexual intercourse, the two previous complain maybe due to ovulation disorders.9.8% of the women had polycystic ovary syndrome, and it was found that 8.2% and 4.9% suffered from hypothyroidism and prolactinemia respectively. 32.8% of the wives and 34.4% of husbands practiced some sort of exercise such as walking, while 19.7% of both, the wives and husbands used dyes to change their hair color, a factor that may cause infertility. None of the women sample smoked while 75.4% of the male sample was heavy smokers, and half of them (47.86%) smoke more than 3 packs per day. The study also reflected that 93.4% of the infertile husbands preferred to use the hot tube for more than 30 minutes every week, and 86.9% of the husbands work required long standing in hot weather and their official attire was made of material such as light jeans. Therefore the role of the nurse in infertility has evolved into a very specialized filed, the maternity nurse must also be involved in the preconception unit, preparing and delivering health education programs about weight, nutrition, smoking, drug abuse and recreational drug, occupational stress prevention programs and stress management is needed.

[Tahani Bin Aoun and Salma Moawed. **Effects of Environmental, Cultural, and Socioeconomic Factors on Saudi Infertile Couple in Riyadh City**. *Life Sci J* 2012;9(4):4861-4868] (ISSN:1097-8135). http://www.lifesciencesite.com. 731

Key Words: Environmental, Cultural, socioeconomic factors, infertile couples

1. Introduction

Infertility has often been defined as a failure to achieve pregnancy within 1 year of unprotected intercourse. [(1)]. Infertility affect 10% of couples, usually as a result of asymptomatic infection, education, poverty, nutrition, and the pollution are problems that must be tackled.[(2)]

Infertility is affected by many different socioeconomic factors, cultural, environmental, and socioeconomic factors, environmental factors play a major role of infertility[(2)] The reproductive system is particularly vulnerable to the effect of the environment. This may be due to dramatic events such as major disasters that may be man-made or natural. However, the greatest number of reproductive failure worldwide are due to endemic conditions of the environment, which are greatly influenced by cultural, religious, political, and socioeconomic factors.[(2)] Which including the following categories: physical, psychological and chemical (occupational e.g. social events, welding, agriculture, biomedical research and laboratories, lifestyle (e.g. alcohol, caffeine, smoking) and inadvertent (e.g. air, water and food pollution)[(3,4)]

On the other hand psychological stress in work place becoming an important factor in infertility. [(5,6)] It was found that there was a significant dose response relationship between level of perceived job stress and poor sperm quality. Prevalence rate for Infertility to the 6.1 million people in 1997 5.3

million Americans or 9% of reproductive age (American Society for Reproductive Medicine) in Saudi Arabia Extrapolated Prevalence 578,511, Population Estimated Used 25, 795, 9382. [7]

Infertility is now recognized as a public health issue, in Saudi Arabia must not ignore the socio cultural and environmental factors given adequate attention particularly in respect of its diagnosis, treatment and prevention. This requires a global infertility approach, the implementation of any program aimed at solving couples in infertility in that are prevalent in the society. The most cost – effective approach to solving infertility problem is prevention and education. The maternity nurse which is working in maternity field or other agencies should be sensitive to the previous issue and share in health education program , planning and implementation regarding to all the previous factors which predisposing in the infertility problem and may be this program produce a rapid improvement in women's health and successful reproductions.

Objectives:

Assess of environmental, cultural, and socio-economic factors thought to effect human fertility among couple attending in-verto fertilization clinic in three hospitals in Riyadh city (King Saud Medical Complex, King Fahad Medical City, and a private hospital).

2. Material and methods:

Setting

This study was carried out at three hospitals in Riyadh city (King Saud Medical Complex, King Fahad Medical City, and a private hospital). This hospital was chosen because of availability of in vitro fertilization clinic with good number of patient and nursing staff with good facilities.

Study subject:

The population of this study consisted of Saudi couple coming for IVF process**.** They like to participate voluntarily and share in this study were included. After consent a paper for an agreement.

Tool used for data collection

The instrument was adapted by the researcher from the Sher institutes for reproductive medicine in Las Vegas, Nevada, United States in 1/6/2006 and modified accordingly to the situation.[8]a pilot study was conducted to evaluate the tool.

Instrument

Data were collected by one instrument , consisted of six parts .the first part include questions related to the information about the socio-demographic data of Saudi infertile couple such as age, educational level, occupational status .

The second part the questioners aimed at assessing the obstetrical history, and include 5 questions.

The third part of the questioners aimed to assess gynecological history, and include a total number of 10 questions

The fourth part include past medical conditions which the couple are having a history of any condition.

Fifth part include social history of the couple, the sixth part include the life style and eating habits.

To measure the face validity of the instrument a group of judges was selected. The group included three faculty members from King Saud University. One faculty member from college of applied medical science, one professor for maternity and gynecological nursing for maternity and gynecological nursing, one statistician, to check clarity, simplicity, relevance to purpose of study.

Method of Data Collection:

The data collection covered a period of nearly 3 months; starts from 1st February till end of April 2010. It was carried out five day/week. I information was obtained by couple interview, review of medical records to check all the information.

Each couple was interviewed by the investigator a brief explanation of the purpose of the study was given to the couple. They were assured that all information gathered was confidential.

Statistical Analysis:

Statistical analysis was done using SPSS package version 11.

Nominal and ordinal variables were presented as frequency and percentage.

Quantitative (interval/ratio) variables were presented as range, mean and standard deviation.

Correlation between ordinal variables was conducted using Spearman rank correlation coefficient with 5% level of significance

3. Results:

Finding of the following study are organized according to research design and presented in four Parts. The first part includes the general characteristics, the second part include obstetrical and gynecological history, the third part present findings related to medical history and finding related to analysis of social history, and the forth part present life style and eating behavior.

General Characteristics:

Finding in this section presents the socio demographic characteristics of the study sample.

Socio- Demographic data:

The demographic characteristics of the study sample are detailed in table1 The table reveals that nearly half percent (49.2%) of the women were aged

between 26-30 years and only 3.3% of the respondents were aged below 20 years and 4.9 % their age 36 and above. while 33.4 % of the husband their age ranged between 31- 35 and 14.8% their age above 41. the difference is statically significant ($P < 0.05$)

With the respect of the education more than tow third of wife completed secondary school and above (67.2%) while 44% of their husband the same previous level of education.

In relation to wives occupation it was found that most of the wives are house wives (80.3%) while 95.1% of husbands occupation was military employee more than half of the couple their duration marriage was 5-10 years (Average7.4±4.7).

Obstetrical and Gynecological History:

The response to all items related to obstetrical history, yielding total mean score How long have you been trying to have a baby (5.8±3.3 years) these are presented in table 2, these results indicated that the range 1-18 years. The table also reflects that more than quarter 78.7% of the sample hasn't any pregnancy.

Regarding the gynecological status, Table 3 it was observed that 80.3% with regular period, 78.7% the amount of menstrual flow ranged from medium in amount to heavy flow, 85.2% are not taking medications to bring on their period. 54.1% are experiencing pain with their menstruation , 60.6% are getting moderate to sever pain, respectively 54.5% of women's menstrual pain relived without use of any medications,78.8% menstrual pain starts with the bleeding, 51.5 of menstrual pain persist more than 48 days, 37.7 are having pain with ovulation ,78.7 are not experiencing pain with sexual intercourse and 61.5 of women's who had pain during sexual intercourse are mostly exterior, 77% are not experiencing vaginal discharge and 64.3 of those who experience vaginal discharge have no itching and 85.7 has no odor, 90.2% are not experiencing milk discharges from breasts, 96.7% don't use OCP or IUD.

In relation to sexual practice / week it was found that (42.6%) had 3 times and 36% practice sexual relation for one or two per week, while 21.4% of the couple practice more than 4 times per week.

Among those women 8.2 % use post coital vaginal douches as a method to clean herself while 91.8% women don't do it. More over 9.8% of the women use lubricant during sexual act.

Medical History

Table 4 shows that only 9.8, 13.1, 4.9, 9.8, 8.2, 4.9, 4.9 and 6.6 had PMS, Facial hair, Acne, Fibroid, Ovarian cyst, Hypothoiroidesm, Prolacteinemia, Overweight and Underweight respectively.

Table 5 demonstrates the past medical history of husbands was attended with their wives to in vitro fertilization clinic. Most of them haven't any major health problem only 4.9% had lung problems and underweight respectively.

Table 6 represents the distribution of the infertile wife and their husbands according to their life styles. The table reflects that more than one third of either group (32.8% and 34.4% did exercises at sometimes but not always and p value between wife and husband was statically significant Spearman rank correlation coefficient (r_s=0.41) p<0.001*.

In relation to hair dyes it was found that 19.7% of wives and 18% of husbands always used dyes Spearman rank correlation coefficient (r_s=0.47 p<0.001 *Significant).

In relation to radiation effect on infertile couple it was found that 18% of female was used microwave always, while 39.3% of their husbands used it sometimes. Spearman rank correlation coefficient (r_s=0.72 p<0.001*Significant).

As regard to air pollution the study reflect that (14.8%) used insect killer always while 42.6% of those women used it sometimes, on the other hand their husband used insect killer always (14.8) and 32.8 % used it sometimes Spearman rank correlation coefficient (r_s=0.75 p<0.001*Significant) .as a Saudi couple they used to use smokes to give a nice odor to environment, it was found that 36.1% of wife and 32.8% of husband often use this smoke.

As regard to exposure to stress from family or relative and work place it was found that 18% of the wives express they exposure to stress always and 26% mentioned they exposed to stress sometimes on the other hand 19.7% of their husband mentioned they have stress.

The same table reflects that more than one third from wife and husband had disturbance in sleeping pattern (31.1%and 37.7%) respectively.

In relation to nutritional intake the table also reflect that the women mentioned that they eat salad, beverages, meats, vegetables, fast food, junk food and dairy products sometimes not always(37.7%, 44.3, 55.7%, 52.5%, 52.5%, 50.8%, 32.8%).

Table 7 shows that less than one third (24.6) of infertile husband nonsmoker while more than two third (75.4%) smoker, nearly half percent smoke more than 3 packets.

Table 8 shows that more than half percent from the wife and husband used to have coffee every day (60.7%) and 52.5% respectively) and 54.1% of wife and 75% husband respectively they drink more than three cups (N.B small cup nearly 100 ml of coffee).

Table 9 it was observed that more than half of the sample (husband) likes to use hot tub bath for at

least once/week. (93.4%). As majority of this sample employee in military agencies so they wear clothes look tight and made from material like jeans (86.9%).

(Table 1): Frequency and percentage distribution of demographic characteristics (N= 61)

Distribution of the studied cases according to age (years).				
Age group	Wife		Husband	
	No.	%	No.	%
<20	2	3.3	0	0.0
20-25	13	21.3	1	1.6
26-30	30	49.2	11	18.0
31-35	13	21.3	21	34.4
36-40	3	4.9	17	27.9
41-45	0	0.0	9	14.8
46-50	0	0.0	2	3.3
Distribution of the studied cases according to educational level				
Educational level	Wife		Husband	
	No.	%	No.	%
Illiterate	6	9.8	0	0.0
Elementary	9	14.8	7	11.5
Intermediate	5	8.2	10	16.4
High school	26	42.6	26	42.6
University above	15	24.6	18	29.5
Distribution of the studied cases according to occupation.				
Occupation	Wife		Husband	
	No.	%	No.	%
House wife	49	80.3	-	-
Student	1	1.6	0	0.0
Employed	11	18.1	58	95.1
Retired	0	0.0	3	4.9

How long currently married (years)?

# OF YEARS	No.	%
<5	17	27.9
5-10	31	50.8
>10	13	21.3
Total	61	100.0

Table (2): Distribution of the studied cases according to obstetrical history.

Obstetrical history	No.	%
Duration since starting treatment		
1	4	6.6
2	4	6.6
3	11	18.0
4	5	8.2
5	5	8.2
6	11	18.0
7	6	9.8
8	6	9.8
>8	9	14.8
Have you ever been pregnant before?		
Yes	13	21.3
No	48	78.7

Table (3): Distribution of the studied cases according to gynecological history

Gynecological history	No.	%
Are your periods regular?		
Yes	49	80.3
No	12	19.7
Amount of bleeding		
Light	13	21.3
Medium	31	50.8
Heavy	17	27.9
Have you ever needed medication to bring on your period?		
Yes	9	14.8
No	52	85.2
Pain with menstruation?		
Yes	33	54.1
No	28	45.9
Degree of pain (n=33)		
Mild	13	39.4
Moderate	14	42.4
severe	6	18.2
Pain relieved by over the counter medications (n=33)		
Yes	15	45.5
No	18	54.5
Pain starts with the onset of bleeding (n=33)		
Yes	26	78.8
No	7	21.2
Pain persists more than 48 hours (n=33)		
Yes	17	51.5
No	16	48.5
Do you have pain with ovulation?		
Yes	23	37.7
No	38	62.3
Do you experience pain with sexual intercourse?		
Yes	13	21.3
No	48	78.7
Pain with sexual intercourse (n=13)		
Mostly exterior	8	61.5
Mostly interior	5	38.5
Are you experiencing vaginal discharge?		
Yes	14	230
No	47	77.0
Associated with itching or burning? (n-14)		
Yes	5	35.7
No	9	64.3
Associated with unusual odor? (n-14)		
Yes	2	14.3
No	12	85.7
Do you experience milk or discharge from your breasts?		
Yes	6	9.8
No	55	90.2
Have you ever used an IUD? OCP?		
Yes	2	3.3
No	59	96.7

Intercourse/week

Frequency	No.	%
1	11	18.0
2	11	18.0
3	26	42.6
4	7	11.5
5	4	6.6
6	2	3.3
Postcoital vaginal douche		
Yes	5	8.2
No	56	91.8
Lubricant		
Yes	6	9.8
No	55	90.2

Table (4): Distribution of wives according to their past medical history

History of medical condition	No.	%
PMS		
Yes	6	9.8
No	55	90.2
Facial hair		
Yes	8	13.1
No	63	86.9
Acne		
Yes	3	4.9
No	58	95.1
Fibroid		
Yes	3	4.9
No	58	95.1
Ovarian cyst		
Yes	6	9.8
No	55	90.2
Hypothyroid		
Yes	5	8.2
No	56	91.8
Prolactinemia		
Yes	3	4.9
No	58	95.1
Overweight		
Yes	3	4.9
No	58	95.1
Underweight		
Yes	4	6.6
No	57	93.4

Table 5 Distribution of husbands according to their medical history

History of medical condition	No.	%
Hypothyroid		
Yes	1	1.6
No	60	98.4
Lung condition		
Yes	3	4.9
No	58	95.1
Hypertension		
Yes	2	3.3
No	59	96.7
Hepatitis		
Yes	1	1.6
No	60	98.4
DM		
Yes	1	1.6
No	60	98.4
Overweight		
Yes	1	1.6
No	60	98.4
Underweight		
Yes	3	4.9
No	58	95.1

Table 6) Distribution of the studied cases according to their life style and eating habits. (*Wives, Husbands*)

Item	*Wives*								*Husband*								*Spearman rank correlation coefficient r_s=*	*P. value*
	Always		*Often*		*Sometimes*		*Seldom*		*Always*		*Often*		*Sometimes*		*Seldom*			
	N	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*	*N*	*%*		
1. Exercise	*16*	*26.2*	*8*	*13.1*	*20*	*32.8*	*17*	*27.9*	*13*	*21.3*	*8*	*13.1*	*21*	*34.4*	*19*	*31.1*	*0.41*	*<0.001*
2. Hair Dies	*12*	*19.7*	*8*	*13.1*	*21*	*34.4*	*20*	*32.8*	*11*	*18.0*	*5*	*8.2*	*20*	*32.8*	*25*	*41.0*	*0.47*	*<0.001*
3. Environmental *A. radiation.*																		
Microwave exposure	*11*	*18.0*	*4*	*6.6*	*20*	*32.8*	*26*	*42.6*	*11*	*18.0*	*4*	*6.6*	*20*	*32.8*	*26*	*42.6*	*0.72*	*<0.001*
B .air pollution																		
1. Insect killer	*9*	*14.8*	*8*	*13.1*	*26*	*42.6*	*18*	*29.5*	*9*	*14.8*	*6*	*9.8*	*23*	*37.7*	*23*	*37.7*	*0.75*	*<0.001*
**2. Smokes*	*9*	*14.8*	*22*	*36.1*	*21*	*34.4*	*9*	*14.8*	*9*	*14.8*	*20*	*32.8*	*22*	*36.1*	*10*	*16.4*	*0.89*	*<0.001*
4. Stress	*11*	*18.0*	*5*	*8.2*	*26*	*42.6*	*19*	*31.1*	*12*	*19.7*	*6*	*9.8*	*23*	*37.7*	*20*	*32.8*	*0.51*	*<0.001*
5. Sleep	*9*	*14.8*	*19*	*31.1*	*25*	*41.0*	*8*	*13.1*	*8*	*13.1*	*22*	*36.1*	*24*	*39.3*	*7*	*11.5*	*0.78*	*<0.001*
6. Nutrition																		
a. Eat salad	*14*	*19.7*	*12*	*23.0*	*24*	*39.3*	*11*	*18.0*	*12*	*19.7*	*13*	*21.3*	*23*	*37.7*	*13*	*21.3*	*0.77*	*<0.001*
b. Beverages	*7*	*11.5*	*10*	*16.4*	*28*	*45.9*	*16*	*26.2*	*7*	*11.5*	*12*	*19.7*	*27*	*44.3*	*15*	*24.6*	*0.66*	*<0.001*
c. Meats	*8*	*13.1*	*12*	*19.7*	*31*	*50.8*	*10*	*16.4*	*5*	*8.2*	*13*	*21.3*	*34*	*55.7*	*9*	*14.8*	*0.92*	*<0.001*
d. Vegetables	*8*	*13.1*	*10*	*16.4*	*32*	*52.5*	*11*	*18.0*	*8*	*13.1*	*10*	*16.4*	*32*	*52.5*	*11*	*18.0*	*0.87*	*<0.001*
e. Fast food	*8*	*13.1*	*9*	*14.8*	*30*	*49.2*	*14*	*23.0*	*5*	*8.2*	*11*	*18.0*	*32*	*52.5*	*13*	*21.3*	*0.86*	*<0.001*
f. Junk food	*8*	*13.1*	*7*	*11.5*	*33*	*54.1*	*13*	*21..3*	*8*	*13.1*	*10*	*16.4*	*31*	*50.8*	*12*	*19.7*	*0.77*	*<0.001*
g. Dairy products	*11*	*18.0*	*14*	*23.0*	*21*	*34.4*	*15*	*24.6*	*9*	*14.8*	*16*	*26.2*	*20*	*32.8*	*16*	*26.2*	*0.53*	*<0.001*

**in Saudi culture always people burn something have good smell and result lead to smoky atmosphere*

Table 7 Distribution of infertile husband according to smoking pattern

Item	No	%
Non smoker Smoker	15 46	24.6 75.4
No of packs/day		
One Two Three	9 15 22	19.59 33.33 47.82

Table 8 Distribution of infertile couple to caffeine intake

Wife			Husband		
Item	No	%	Item	No	%
Yes	37	60.7	Yes	32	52.5
No	24	39.3	No	29	47.5
How many cup /day No 37			How many cup /day No 32		
	No	%		No	%
1 2 3 >3	2 6 9 20	4.20 16.21 24.32 54.05	1 2 3 >3	2 3 3 24	6.25 9.38 9.38 75

Table 9 Distribution of the infertile husband according to tub bath and clothes

Item	No	%
Hot tub		
Yes	57	93.4
No	4	6.6
Tight cloths		
Yes	53	86.9
No	8	13.1

4. Discussion:

Women health care is concerned with most areas of priorities for health promotion. However, emphasis in accordance with the individuals characteristics and stages of life. nurses play an important role in promoting health through teaching and counseling that assist clients to make and important healthy chimes in their daily lives many studies have demonstrated that teaching and counseling by health profanes is effective in changing people's erroneous health behaviors.

Infertility is a complex problem with many personal variables for the couple involved. [7]

The age of infertile women in this study was between 26-30 years mean years of marriage was 4-7 years and most of them housewife. Fertility declines with age ,it is typically declines during mid to late thirties as the number of egg declines and ovaries produce less estrogen and progesterone and egg become resistant to fertilization and tended to have more chromosomal abnormalities [3]

Moreover, for the previous factors they are worry and came to see a specialist to do IVF procedure.

Finding from the recent study indicate that most of infertile women their blood loss during menstruation either it moderate amount or heavy (78.7%) and 51.5% have dysmenorrhia and 37.7% having pain during sexual intercourse, all the previous complain may be due ovulation disorder [8].

It was observed that 9.8% of the infertile women have polycystic ovary syndrome and it is considered as the most common endocrine disordered in women and cause of infertility on the other hand, it was found that 8.2% and 4.9% had the hypothyroid, prolactinemia and overweight respectively. Hypothyroidism can have a notable effect on infertility and women may present with infertility caused by thyroid disease. Hypothyroidism is associated with a rise in serum prolactinemia levels. In addition, prolactin level can be elevated by stress and can vary daily. [9]

Table 6 portrays the relation between the life style and infertile couple. It was found that 32.8% wife and 34.4% of husband practice exercises such as walking sometimes, while 19.7% nearly from wife and husband use die to change color of hair always this may.

Consider as one of drugs and environmental chemicals may lead to problem during time of ovulation or cause. Injury usually kills the affected embryo 18% of both wife and husband always use microwave for cooking and warming food, 42.6% of wife, use insect killer and 32.8% of husbands use the same method often. Smokes (some brown wood had nice smell when burn) 36.1% of wife use about twice per day and when guests came to spread this nice odor among home rooms and as traditional method for welcome by gust. Therefore, they use it; also 32.8% of husbands use this method. [10]

It appears that some women with high stress levels may have hormonal changes and interfere with ovulation. In addition, in men, stress may be one of many factors responsible for decrease sperm productions. Moreover, infertile couples experience chronic stress each month .first hoping that they will conceive and then dealing with Disappointment if they do not, treatment can place additional stress on couple the medical evaluation to determine the source of infertility and the treatment interventions can create tremendous finical and emotional stress couples. Some stress research has show that women

undergoing infertility treatment experience an equal or higher level of stress of these faced with life threaten illness of cancer or heart disease [11]

Infertile female and male in this study had more disturbance in sleep, nearly one third of them they mentioned that they sleep only from 2-3 hours per 24/hours this may be due to many environmental factors or psychological disturbance or for male part they going outside home and sit with friend all night same thing like escaping from stress environment at home. On the other hand women are more anxious as Saudi culture gives great importance to pregnancy and chilled bearing, also women was afraid from her husband to married from another one [12, 17, 18, 19]

The results of this study reveled that only 19.7% of infertile couple eat salad once/day and 13.1% eat once cooked vegetables and 13.1% of women and 8.2% of male eat meats once per day. It observe from previous analysis that the intake is less than daily required as vitamin C is important in sperm production and keep the sperm from clumping and make as them more mature. Also Vitamin E needed for balanced hormone production been know as agent and increase sperm count, the sources of vitamin E manly dark green leafy vegetables and whole grains, zinc is another mineral its deficiency lead to delayed sexual maturation and infertility so, a nutritional education is important to be done by the nurses or nutritionist [13]

The results of the study showed that no infertile women were smoke while 75.4% of infertile male heavy smoker and nearly half percent (47.86%) smoke more than 3 packs per day. This may lead to poor sperm quality or quantity and increase risk of improved sperm production [14, 15]

This study reflect that more than half of the sample either infertile their caffeine intake were more than 3 cups per day as same studies have shown a prolonged waiting time to conception among women with higher caffeine intake (equivalent of more than tow cups of coffee per day) effect of caffeine use on male reproductive ability have not been well studied and cannot be evaluated. [6, 16]

Another factor this result reflected on the infertile husband it was observed that 93.4% prefer to use hot tub bath once a week for more than 30 minutes per week , and as they work required long standing in hot weather and with tight clothes their official clothes made from a material such light jeans (86.9%). All this personal and occupational factors may all contribute to infertility and risk abnormal sperm motility [20, 21, 22]

Therefore, the role of nurse in infertility has evolved into a very specialized filed. The maternity nurse must also involve in pre conception unit, health education program about weight, nutrition, smoking, given up drug abuse or recreational drugs, occupation of health prevention program, stress management are needed.

Nurses should be involved in educational forums that will specially address the specialty of infertility of infertility nursing and should also have access to current medical information that is relevant to the field of infertility.

References:

1) Lowdermilk D.L., Perry S.E.: Study Guide For Maternity & Women's Health Care, 10th Edition. *Mosby;* (March 9, 2011) ISBN-10: 0323074308, ISBN-13: 978-0323074308
2) Younglai EV, Holloway AC, Foster WG. (2005): Environmental and occupational factors affecting fertility and IVF success. *Hum Reprod Update*. 2005 Jan-Feb;11(1):43-57. Epub 2004 Dec 15.
3) Cocco P (2002): On The Rumors About The Silent Spring. Review Of The Scientific Evidence Linking Occupational And Environmental Pesticide exposure To Endocrine Disruption Health Effects. *Cad. Saúde Pública, Rio de Janeiro*, 18(2):379-402, mar-abr, 2002
4) Lindbohm ML (1999): Women's Reproductive Health: Some Recent Developments in Occupational Epidemiology. *Am J Ind Med.* Jul;36(1):18-24.
5) http://www.rightdiagnosis.com/i/infertility/stats-country.htm
6) Bolúmar, F., Olsen, J., Rebagliato, M. and Bisanti, L. (1997): Caffeine intake and delayed conception: A European multicenter study on infertility and subfecundity. European study group on infertility subfecundity. *American Journal of Epidemiology*, 145(4), 324-334.
7) Cooper-Hilbert, B (2001): Helping Couples Through The Crises Of Infertility. Clinical Updates: *The American Association of Marriage and Family Therapy* 3, 1-6.
8) Waller KG, Show RW (1995): Endometriosis Pelvic Pain and Psychological Functioning. *Fertil Steril.* Apr;63(4):796-800.
9) Elizabeth A. Gangar, Vicki Allanach: Gynaecological Nursing: A Practical Guide. *Churchill Livingstone* (6 Jun 2001) ISBN-10: 0443062021, ISBN-13: 978-0443062025
10) Bourgeois F. John, Bray Megan J., Matthews Catherine H.: Obstetrics and Gynecology Recall, 2007. Third Edition, *Williams And*

Willing U.S.A. ISBN-10: 0781770696, ISBN-13: 978-0781770699

11) Kathryn J Watkins, Tracy D Baldo: The Infertility Experience: Biopsychosocial Effects and Suggestions for Counselors. *Journal of Counseling and Development*: JCD. Alexandria: Fall 2004. Vol. 82, Iss. 4; pg. 394, 9 pgs.
12) Al-Abdulkareem AA, Balla SG (1998): Consanguineous Marriage in Urban Area of Saudi Arabia, Rates and Adverse Health Effect on the Offspring .*Journal Of Community Health*. Feb; 23(1):75-83.
13) Phyllis Balch, James Balch (2000): Prescription for Nutritional Healing (Prescription for Nutritional Healing: A Practical A-To-Z Reference to Drug-Free Remedies). *Avery*; 3rd edition. ISBN-10: 1583330771, ISBN-13: 978-1583330777.
14) Jung A, Schill WB (2000): Male infertility. Current life style could be responsible for infertility. *MMW Fortschr Med* 2000;142:31-3.
15) Kapawa A, Giannakis D, Tsoukanelis K, Kanakas N, Baltogiannis D, Agapitos E, Loutradis D, Miyagawa I, Sofikitis N. (2004): Effects of paternal cigarette smoking on testicular function, sperm fertilizing capacity, embryonic development, and blastocyst capacity for implantation in rats. *Andrologia.* Apr;36(2):57-68.
16) Gray RH, Fuentes A. (1994): Infertility Epidemiligy. *Contemp Obstet Gynecol* 39:70-81.
17) Hassan MA, and Killick SR (2004): Negative lifestyle is associated with a significant reduction in fecundity. Fertile Steril 2004 Feb;81(2):384-92.
18) Klonoff-Cohen H, Chu E, Natarajan L and Sieber W (2001): A prospective study of stress among women undergoing in-vitro fertilization or gamete intrafallopian transfer. *Fertil Steril*. Oct;76(4):675-87.
19) Eugster A and Vingerhoets AJJM (1999): Psychological aspects of in-vitro fertilization: a review. *Social Science & Medicine* 48:575-589.
20) McElgunn B (1998): Reproductive and developmental hazards in the workplace. *Clinical Excellence for Nurse Practitioners: the International Journal of NPACE* [2(3):140-145].
21) Bigelow PL, Jarrell J, Young MR, and Keefe TJ And Love EJ (1998): Association Of Semen Quality And Occupational Factors: Comparison Of Case-control Analysis And Analysis Of Continuous Variables. *Fertil Steril*. Jan;69(1):11-8.
22) Campagna C, Sirard MA, Ayotte P and Bailey JL (2001): Impaired maturation, fertilization, and embryonic development of porcine oocytes following exposure to an environmentally relevant organ chlorine mixture. *Biology of Reproduction* 65, 554–560 (2001).

12/12/2012

Personal Preference and Perceived Barriers toward Disclosure and Report of Incident Errors among Healthcare Personnel

Shereen Ragab Dorgham and Lobna Khamis Mohamed

Nursing Services Administration Dept, Nursing Faculty, Tanta University
d_org_ham7@hotmail.com and dr.lobnakhamis@yahoo.com

Abstract: Background: Despite the best intentions of caregivers, medical errors occur frequently. Each year thousands of injuries and deaths in hospitals result from medical errors. Reporting and disclosure about incidents errors is fundamental to error prevention. **Aim**: The aim of this research was to assess the personal preference and perceived barriers toward disclosure and report of incident errors among healthcare personnel. **Design**: A descriptive cross-sectional design was used for this research. **Setting**: The study was conducted at two hospitals' namely; King Fahd Hospital of the University (KFHU) in Saudi Arabia and El-Behara Hospitals in Egypt in Intensive Care Units (ICUs) and surgical department. **Sample**: The sample included 155 health care professionals (physicians and registered nurses). **Tools**: Two tools were used in this study; the first tool used to assess perceived barriers to medical errors and the second tool used to assess personal preference about which incidents to be disclosed and to whom disclose or report errors. **Results**: The current study demonstrated underreporting of adverse events by both nurses and physicians due to administrative barrier which considered as a major barrier. Majority of total sample preferred to disclose errors with near miss, followed those who don't prefer to disclose any errors then those preferred to disclose errors with minor harm. Furthermore, the majorities of participants did not prefer to disclose errors for patients or their families and did not prefer to report errors for colleges, head nurses or chief executive officer. **Conclusion**: When errors are not reported, the potential to avoid future preventable errors is greatly reduced. Thus, sustained and collaborative efforts to reduce the occurrence and severity of health care errors are required so that safer, higher quality care results. **Recommendations:** The results recommended the needs to improve healthcare professionals' education, training, and practice in disclosure, and health care institutes should establish non-punitive policies of error reporting and implement full disclosure policies.

[Shereen Ragab Dorgham and Lobna Khamis Mohamed. **Personal Preference and Perceived Barriers toward Disclosure and Report of Incident Errors among Healthcare Personnel**. *Life Sci J* 2012;9(4):4869-4880] (ISSN:1097-8135). http://www.lifesciencesite.com. 732

Keywords: Incidents errors, reporting, disclosure and healthcare personnel

1. Introduction

Medical errors that injure or cause death in patients have become a significant and costly problem prompting governmental and regulatory agencies, health care organizations, and private industry to seek solutions to reduce errors and minimize their effect on individuals while limiting their cost[(1)]. Despite the best intentions of caregivers, medical errors occur frequently. Each year thousands of injuries and deaths in hospitals result from medical errors[(2)].

Error-free performance is a standard expected from all healthcare professionals. However, health systems and personnel are not infallible; errors are made, with high human and economic costs. Some errors have devastating consequences; others do not. Adverse events are visible manifestations of errors, and most represent unintended errors of omission (usually) or commission[(3)]. Learning from both adverse events and near misses (i.e., an event/occurrence where harm to the patient was avoided) are essential for improving the quality of care[(4)].

Patient safety is a central concern of current healthcare delivery systems and quality[(5)]. Contrary to commonly held perceptions, medical errors are generally not the result of individual misconduct; they are caused by failures in the health care systems and organizations that we create[(6)]. However, Physicians and nurses play a pivotal role in the identification, prevention, and reduction of medical errors and promotion of patient safety[(1)].

Commonly, neither health care professionals nor health care organizations counsel others when a mishap occurs, nor do they share what they have learned when an investigation has been carried out. Consequently, the same mistakes occur repeatedly in many settings and patients continue to be harmed by preventable errors[(7)]. Errors in the health care system have been major public health problems which caused due to a diverse interaction of human behavior, socio-cultural aspects, technical aspects of the system, as well as a range of system weaknesses[(8)].

Incident is an unplanned and often destructive event that disrupts the administration, development or

continuation of the work. Incident is caused by unsafe tasks or working in unsafe conditions or a combination of the two, because of the lack or weakness in its detection, or due to some failures in risk control systems[9]. Reporting about incidents errors is fundamental to error prevention. Concealing a medical error may violate ethical codes. Moreover, the process of reporting errors requires courage, composure, communication skills, and a belief that the importance of telling the truth. Nevertheless, many possible reasons explain why medical personnel may not be forthcoming with the truth[7,10]. Actually, reporting an adverse event is a difficult and fearful event for healthcare providers. It may not be easy, but ethically it's the right thing to do. In fact, the process of disclosing is an ethical and legal obligation that provides essential information to patients and families. Nonetheless, errors can happen at every level of the healthcare continuum. There is a significant gap between what is expected and what actually occurs in current practice; therefore increasing attention to disclosing harmful events is inevitable[11].

Individual physicians, nurses, pharmacists, and other health care practitioners inevitably make mistakes in judgment, overlook a symptom, and fail to use medication or equipment properly, or misinterpret a finding. Furthermore, the devices, machines, medications, and other equipment used in treating and diagnosing patients play an important role in determining health care safety and can also serve as a source of error[12,13].

Many errors go unreported by health care workers[3]. The major concern of them is that self-reporting will result in repercussions. Providers' emotional responses to errors inhibit reporting, yet some are relieved when they share the events of the error with peers or others[14,15]. Health care professionals report feeling of worried, guilty, and depressed following serious errors, as well as being concerned for patient safety and fearful of disciplinary actions[16,17]. Therefore, they choose not to acknowledge or document errors. In other cases, errors are discussed only behind closed doors between providers and administrators; patients and families aren't told when errors have occurred, or that corrective actions are needed. Thus, certain kinds of errors re-occur, and the risk for patient harm increases[17].

Disclosure of errors is required per professional, legal and regulatory standards[18]. Consequently, the process of reporting and disclosing medical errors requires agreement among health care professionals about what constitutes an error; how errors should be reported; and when, how, and by whom they should be disclosed[19].

The lack of standardization in the information that is reported and collected makes comparisons and trending more difficult in preventing future errors[20]. When health care providers do not recognize, report, or disclose errors, they fail to act in the best interest of the patient. This failure compromises patient autonomy and informed decision-making. The failure to report and disclose errors also compromises the principles of beneficence, fidelity, and justice[21]. Research has revealed that errors are a growing problem in the family practice setting, and upon discharge from the hospital[22].

Indeed, health care organizations are morally obligated to develop and implement a disclosure policy that promotes open and honest communication in order to honor and respect patients, and to maximize benefits, reduce harm, reflect honesty and truthfulness in the patient/clinician relationship[22,23]. Failure of professionals to communicate effectively, and to honestly admit to the error in a timely manner, can potentially undermine the hospital's reputation and heighten the risk of litigation. Health care providers may experience a certain relief when disclosure policies have been crafted and are in place[24].

Accordingly, reporting (providing accounts of mistakes) and disclosing (sharing with patients and significant others) actual errors and near misses provide opportunities to reduce the effects of errors and prevent the likelihood of future errors by, in effect, warning others about the potential risk of harm[25]. Reporting reduces the number of future errors, diminishing personal suffering and decreasing financial costs. In contrast, disclosure is thought to benefit patients and providers by supplying them with immediate answers about errors and reducing lengthy litigation. Although clinicians and health care managers and administrators feel uncomfortable with disclosure, disclosure is a duty[26].

Consequently, there are no easy road maps for providers who face a complex problem like medical error disclosure or report. Errors can trigger feelings of shock and anxiety among all parties involved. Recognizing the preferences and perceptions of health professionals who will implement this system is mandatory for its success. Therefore, this study aimed to assess the personal preference and the perceived barriers of health care personnel towards disclosure /reporting of incident/error.

Research's Questions

Three research's questions were asked about:

- What was the relation between demographic data of healthcare personnel and their perceived barriers to medical errors?
- Which type of errors the healthcare personnel preferred to report?

- To whom the healthcare personnel preferred to disclose or report errors?

2. Material and Methods

Design: A descriptive cross-sectional study design was used.

Setting: This study was conducted at two hospitals' namely; King Fahd Hospital of the University (KFHU) in Saudi Arabia and at El-Behara Hospitals (Abohomos, Kafr Eldawar, Etay El Baroad, El Delangat and Hosh Essa) in Egypt in Intensive Care Units (ICUs) and surgical departments.

Sample: 155 health care professionals participated in the study as 52 physicians (consultant and residents) and 103 registered nurses (head nurses and supervisors) worked in the above mentioned settings. Two tools were used in this study.

Tool I: Perceived Barriers' Assessment to Medical Errors.

It consisted of two parts. The first part included demographic data of healthcare personnel such as age, sex, position, work area, working hours/week and years of experience. The second part was developed by **Wakefield et al (2000)**[27] and modified by the researchers based on the recent literature to be 29 items categorized under four sections; fear (12 items), understanding (7 items), administrative barriers (5 items) and burden of effort (5 items). The subjects' responses were represented in five points Likert Scale ranging from 5= strongly agree to 1= strongly disagree.

Tool II: **Personal Preference Assessment.**

It was developed by the researchers which included two parts; part one contained 1 item asked about which incidents to be disclosed with five options for subjects' responses ranged from 1= don't disclose, 2= disclose with major harm, 3= disclose with moderate harm, 4= disclose with minor harm and 5= disclose with near miss.

The second part consisted of two sections. Section one asked about personal preference of healthcare personnel to disclose errors for patient and family (1 item). Section two asked about to whom report errors and contained five items. Two options were allowed for subjects' responses; yes or no.

Both tools submitted to five experts in the different fields of nursing for testing the content and face validity. Necessary modifications were done, included clarification, omission of certain questions and adding others and simplifying work related words.

The researchers used test-retest reliability of nominal data which its value greater than 0.75. Cronbach's coefficient alpha was used to measure internal consistency reliability of tools which greater than 0.7.

Ethical considerations

The research was approved by an ethical committee of Dammam University. The data was collected after the approval of permission from the hospital responsible authorities. Prior to the data collection, informed consent of all participants was obtained. Participants were informed about the purpose of the research study. A pilot study was carried out on 10 healthcare personnel (physicians and nurses) in previously mentioned settings and excluded from the main study's sample.

Statistical analysis

Statistical analysis was carried out using SPSS (version 11.5). Quantitative variables were described by the Mean, Standard Deviation (SD) and the Range (Maximum – Minimum). Qualitative variables were described by proportions and Percentages. Analyzed data was done through the use of two tests; student t-test and ANOVA Spearman's rho is used to measure correlation. Significance level was stated at $\alpha = 0.05$.

3. Results

The overall response rate from 155 participants was 77.5% who agree to participate in data collection. Table (1) shows demographic data of healthcare personnel at El-Behara and Fahd hospitals. at El-Behara participants, (41.1%) were in age group 30 to 39 years, (77.8%) were mainly female with Egyptian nationality, (65%) were nurses, (51.1%) worked in surgical department, (47.8%) had less than 5 years of experience and (67.8%) worked for ≥39 hours/week.

Regarding Fahd participants, (33.8%) were in age group 30 to 39 years, (84.6%) were mainly female, (50.8) had Saudi nationality, (67.7%) were nurses, (60%) worked in surgical department, (29.2%) had 5-9 years of experience and (81.5%) worked for 40-59 hours/week.

Table (2) reveals correlation of demographic data with perceived barriers' subscales to report errors at El-Behara and Fahd hospitals. This study suggested no statistically significant correlation between participants' age and their perceived barriers' subscales in both settings except the understanding barrier at Fahd hospital ($r=0.253$ $p=0.0493$). In relation to years of experience, there were significant correlations with fear ($r=0.158$ $p=0.0499$) and administrative barriers ($r=0.348$ $p=0.0000$) at El-Behara hospitals, also with total perceived barriers ($r=0.225$ $p=0.0049$), as well as in understanding and administrative barriers at Fahd hospital ($r=0.333$ $p=0.0067$, $r=0.326$ $p=0.0081$) respectively, and with total perceived barriers ($r=0.320$ $p=0.0094$). Moreover, there were inverse statistically significant correlations between males and females for all perceived barriers subscales at El-Behara hospitals and positive correlations at Fahd hospital except for fear barrier.

Concerning staff position, inverse significant correlations found between doctors and nurses for all perceived barriers' subscales at El-Behara hospitals except for administrative barrier and positive significant correlations at Fahd hospital except for burden of effort barrier. The table showed no significant correlation between two working areas and their perceived barriers subscales in both settings. Regarding working hours, highly significant correlations presented between different groups of working hours and their perceived barriers' subscales at El-Behara hospitals and no significant correlation found among participants at Fahd hospital.

Table (1): Demographic data of healthcare personnel at El-Behara and Fahd hospitals

Demographic Data	Hospital names			
	El-Behara Hospitals		Fahd Hospital	
	Frequency	%	Frequency	%
Age /years				
20 - 29	32	35.6	13	20.0
30 -39	37	**41.1**	22	**33.8**
40-49	20	22.2	6	9.2
50 – 60	1	1.1	20	30.8
Missing	0	0.0	4	6.2
Total	90	58.1	65	41.9
	Range 20-52 Mean 33.56 & SD 6.72		**Range 26-60 Mean 40.07 & SD 11.43**	
Sex				
Male	20	22.2	10	15.4
Female	70	**77.8**	55	**84.6**
Total	90	58.1	65	41.9
Nationality				
Egyptian	90	**100.0**	2	3.1
Saudi	0	0.0	33	**50.8**
Foreign	0	0.0	30	46.2
Total	90	58.1	65	41.9
Staff Position				
Doctor	31	34.4	21	32.3
Nurse	59	**65.6**	44	**67.7**
Total	90	58.1	65	41.9
Working Areas				
ICU	44	48.9	26	40
Surgical	46	**51.1**	39	**60**
Total	90	58.1	65	41.9
Years of Experiences				
>5	43	**43.0**	11	16.9
5 to 9	27	27	19	**29.2**
10 to 19	20	20	17	26.2
≤20	0	0	18	27.7
Total	90	58.1	65	41.9
	Range 0.3 – 18 Mean 5.91 & SD 4.27		**Range 0.25-32 Mean 11.97 & SD 8.85**	
Working Hours/Week				
≥39	61	67.8	4	6.2
40-59	18	20.0	53	81.5
≤60	11	12.2	8	12.3
Total	90	58.1	65	41.9

Table (2): Correlation of demographic data with perceived barriers' subscales to report errors at El-Behara and Fahd hospitals

Demographic Variables		El-Behara Hospitals					Fahd Hospital				
		Fear Barrier	Underst. Barrier	Admin. Barrier	Burden Barrier	Total PBs	Fear Barrier	Underst. Barrier	Admin. Barrier	Burden Barriers	Total PBs
Age		0.135 0.2046	0.052 0.6236	0.188 0.0757	0.136 0.2017	0.152 0.1521	0.107 0.4136	**0.253** **0.0493***	0.221 0.0869	0.044 0.7350	0.193 0.1361
Experience		**0.158** **0.0499***	0.140 0.0816	**0.348** **0.0000*****	0.092 0.2524	**0.225** **0.0049****	0.200 0.1097	**0.333** **0.0067****	**0.326** **0.0081****	**0.073** 0.5647	**0.320** **0.0094****
Sex	Males	**-2.30** **0.0236***	**-3.37** **0.0011***	**-2.95** **0.0041***	**-3.49** **0.0008****	**-3.75** **0.0003****	0.50 0.6196	**2.47** **0.0163***	**3.60** **0.0006****	**3.58** **0.0007****	2.62 **0.0110***
	Females										
Position	Doctor	**-2.94** **0.0042***	**-2.75** **0.0072***	-1.73 0.0872	**-3.55** **0.0006****	**-3.70** **0.0004****	**2.15** **0.0354***	**2.41** **0.0191***	**3.41** **0.0011****	-0.33 0.7445	2.82 **0.0065****
	Nurse										
Working Areas	ICUs	1.22 0.226	0.06 0.955	-0.36 0.717	-1.19 0.237	0.36 0.722	0.35 0.724	-0.52 0.604	-1.96 0.054	0.31 0.158	0.54 0.860
	Surgical										
Working hours	≥39	**16.39** **0.0000*****	**11.10** **0.0000*****	**5.09** **0.008****	**17.79** **0.0000*****	**24.26** **0.0000*****	0.77 0.466	0.58 0.561	2.45 0.095	0.90 0.413	1.05 0.357
	40-59										
	≤60										

Significant P < 0.05, P < 0.001, P < 0.0001*****

Table (3): Relation of participants' nationality with perceived barriers' subscales to report errors

Perceived Barriers' Subscale	Nationality	N	Mean	SD	F P-value
Fear Barrier	Egyptian	92	63.69	20.42	**8.34** **0.00036*****
	Saudi	33	49.44	24.32	
	Foreigner	30	49.17	21.59	
Understanding Barrier	Egyptian	92	64.36	19.45	3.70 **0.02690***
	Saudi	33	60.18	16.58	
	Foreigner	30	53.92	17.43	
Administrative Barrier	Egyptian	92	84.08	16.24	38.34 **0.00000****
	Saudi	33	64.39	20.11	
	Foreigner	30	53.17	21.52	
Burden of Effort Barrier	Egyptian	92	76.79	20.50	27.76 **0.00000****
	Saudi	33	53.64	19.97	
	Foreigner	30	51.67	17.24	
Total PBs	Egyptian	92	69.62	15.18	21.93 **0.00000*****
	Saudi	33	55.32	14.39	
	Foreigner	30	51.44	15.55	

Significant P < 0.05, P < 0.001**

Table (3) represents the relation of participants' nationality with perceived barriers' subscales to report errors. This table revealed highly statistically significant correlation between three groups of nationalities and their perceived barriers subscales to report errors; fear (r=8.34 p=0.00036), understanding (r=3.70 p=0.02690), administrative (r=38.34 p= 0.0000), burden of effort (r= 27.76 p= 0.0000), as well as total perceived barriers (r= 21.93 p= 0.0000).

Table (4) reveals the perceived barriers to report errors at El-Behara and Fahd hospitals. It can be noticed that administrative barrier had the highest mean scores at both settings (mean=84.50 ± 16.15 & 59.23 ± 21.09) respectively. The next strongest

perceived barrier was burden of effort (mean=77.44 ± 19.96) at El-Behara hospitals and understanding barrier (mean= 57.25 ± 16.90) at Fahd hospital. The weakest perceived barrier was fear at both settings. It was obviously observed that doctors and nurses at El-Behara hospitals have higher mean scores (69.82 ± 15.28) in all perceived barriers' subscales than Fahd hospital (53.70 ± 14.80). Moreover, there were statistical significance differences between two settings in all perceived barriers to report errors; fear, understanding, administrative, burden of effort (t=3.95 p=0.0001, t=2.40 p= 0.01, t=8.45 p=0.0000 & t=7.86 p=0.0000) respectively.

As shown in Figure (1), around half (56.7%) of participants at El-Behara hospitals preferred to disclose errors with near miss and (26.7%) of them didn't prefer to disclose any errors. While considerable percent (29.2%) at Fahd hospitals' respondents didn't prefer to disclose errors and (27.7%) of them preferred to disclose errors with minor harm only.

Table (5) illustrates the personal preference of healthcare personnel to whom disclose/report errors at El-Behara and Fahd hospitals. It stated that the majorities (93%) of participants in both settings did not prefer to disclose errors for patients and their families, as well as did not prefer to report errors for colleges (81.9%), head nurses (59.4%) or chief executive directors (92.3%). On the other hand, 67.7% and 45.6% of participants prefer to report errors for supervisor and physician at Fahd and El-Behara hospitals respectively.

Table (6) represents the correlation between perceived barriers' subscales in El-Behara and Fahd hospitals. It revealed highly statistical significant correlations between all subscales of perceived barriers in both settings except for fear with administrative and burden of work in Fahd hospital no significant correlation was existed.

Table (4): Perceived barriers to report errors at El-Behara and Fahd hospitals

Perceived Barriers Items	Hospital name	Mean	±SD	t-test P value
Fear	**Damanhur**	63.64	20.63	3.95
	Fahd	49.82	22.71	**0.0001*** **
Understanding	**Damanhur**	64.48	19.63	2.40
	Fahd	**57.25**	**16.90**	**0.01***
Administrative	**Damanhur**	**84.50**	**16.15**	8.45
	Fahd	**59.23**	**21.09**	**0.0000*** **
Burden of effort	**Damanhur**	**77.44**	**19.96**	7.86
	Fahd	52.54	18.75	**0.0000*** **
Total PBs	**Damanhur**	**69.82**	**15.28**	6.57
	Fahd	**53.70**	**14.80**	**0.0000*** **

Figure 1: Personal preference to which error to be disclosed

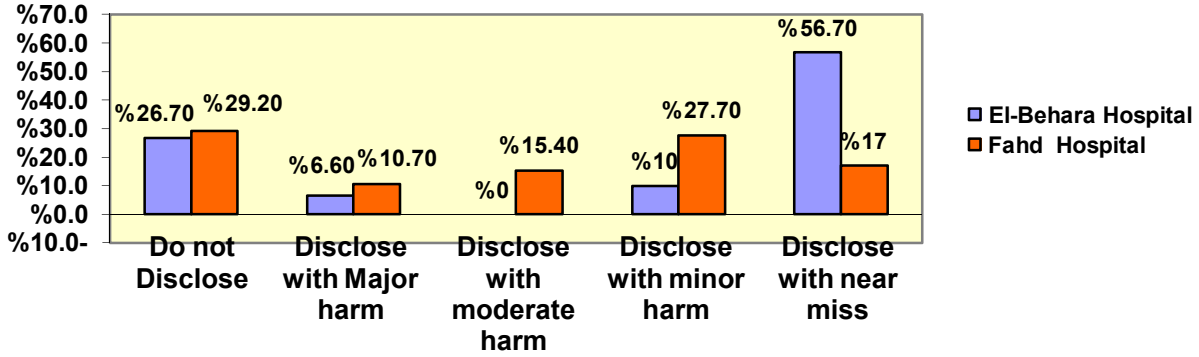


Table (5): Personal preference of healthcare personnel to whom disclose/report errors at El-Behara and Fahd hospitals

Who disclose/report errors	Hospital name	No (%)	Yes (%)
Patient & Family	**El-Behara**	83 (**92.2**)	7 (7.8)
	Fahd	61 (**93.8**)	4 (6.2)
Total		**144 (93)**	**11 (7)**
College	**El-Behara**	74 (**82.2**)	16 (17.8)
	Fahd	53 (**81.5**)	12 (18.5)
Total		**127 (81.9)**	**28 (18.1)**
Head nurse	**El-Behara**	53 (58.9)	37 (41.1)
	Fahd	39 (60)	26 (40)
Total		**92 (59.4)**	**63 (40.6)**
Supervisor	**El-Behara**	83 (**92.2**)	7 (7.8)
	Fahd	21 (32.3)	44 (**67.7**)
	Total	**104 (67.1)**	**51 (32.9)**
Physician	**El-Behara**	49 (54.4)	41 (**45.6**)
	Fahd	37 (56.9)	28 (43.1)
Total		**86 (55.5)**	**69 (44.5)**
CEDs	**El-Behara**	88 (**97.8**)	2 (2.2)
	Fahd	55 (**84.6**)	10 (15.4)
Total		**143 (92.3)**	**12 (7.7)**

Table (6): Correlation between perceived barriers subscales at Damanhur and Fahd hospitals

Perceived Barriers Subscale	El-Behara (n = 90)			Fahd (n = 65)		
	Unders.	Admin.	Burden	Unders.	Admin.	Burden
	r (p)	r (p)	r (p)	r (p)	r (p)	r (p)
Fear	**0.455 0.000*****	**0.395 0.000****	**0.288 0.005****	**0.526 0.000*****	0.225 0.0709	0.026 0.8348
Unders.		**0.274 0.009****	**0.235 0.025***		**0.604 0.000*****	**0.314 0.01****
Admin.			**0.304 0.003****			**0.277 0.025***

Significant P < 0.05*, P < 0.001, P < 0.0001*****

4. Discussion

Although healthcare providers are not expected to make errors, mistakes do occur, and some mistakes have resulted in serious injury or death. Each year, approximately 1.3 million patients are injured because of error during their hospitalization, and more than 100000 deaths due to preventable adverse events occur[28]. Hence, concerns about incidents errors represent a significant threat to patient safety.

To our knowledge, in Arabic Islamic countries no researches study the relationship between perceived barriers of medical errors among nurses and physicians and their demographic characteristics, as well as their personal preference toward which error to be disclosed and to whom disclose/report error. Likewise, the aim of this research is to assess personal preference and perceived barriers toward disclosure and report of incident errors among health care personnel in Egypt at El- Behara Hospitals and in KSA at King Fahd University Hospital.

In this study, no statistical significant differences were found between age and perceived

barriers subscales in both settings except in understanding barrier at king Fahd hospital. These results can be explained as older physicians or nurses were more understanding for meaning of errors and able to determine which error must be reported, as well as, they were mature enough and familiar with the policy of their hospital. On contrary, younger physicians or nurses had lack of knowledge about how, what and whom to report errors. Additionally, reporting errors is not a common practice at El-Behara hospitals in Egypt and not all respondents perceived that an error was serious enough to be reported.

Chiang et al (2006)[29] supported these findings as they found that nurse's demographic characteristics were not related to the perceived barriers. Moreover, this finding is inconsistent with **Blegen et al., (2004)**[30] study who reported that age was negatively correlated to the reporting barriers. One reason for this difference may be that these respondents were much younger and had shorter work experience especially at El-Behara hospitals.

Findings of the current study documented statistical significant differences for respondents' years of experience with fear and administrative barriers at El-Behara hospitals, as well as statistical significant differences with understanding and administrative barriers in Fahd hospital. This congruence between participants' years of experience and administrative barriers in both settings may be due to that more experienced nurses / physicians had more knowledge, skills base and respectful for rules and regulations of hospital[7]. Moreover, the majority of them experienced administrative barrier focusing on the person rather than the system.

In addition, the experienced respondents at El-Behara hospitals revealed increased afraid of blaming, punishment and fear of career threatening disciplinary actions with possible malpractice litigation and liability due to unclear policy about reporting errors. On the other hand, experienced participants at Fahd hospital revealed that they are incompetent and damage their professional reputation when report for error, especially if their hospital didn't provide feedback on reported errors and give exaggerated response regardless severity of error, as well as their leaders may not protect reporters of errors from negative consequences.

Evans et al (2006)[31] research confirms the previous finding and claimed that the frequency of error reporting was found to be higher among nurses with 5-10 years of experience. While, this finding inconsistent with **Mayo and Duncan (2004)**[32] who suggested that a weak relationship between percentage of errors perceived reported and years of RNs' practice. Additionally, another survey reported that nurses with more than 5 years of experience were more likely to believe there was no value in reporting near misses[33]**.** Furthermore, it was found that staff nurse relied on personal experience to estimate medication administration errors on their unit[34].

Moreover at El-Behara hospitals, findings revealed inverse statistically significant correlation between males' and females' respondents for all perceived barriers subscales. This may be attributed to that the majority of the studied sample at El-Behara hospitals was female. Female respondents were more likely than male participants to feel guilty and angry at themselves, and be afraid of accusations of malpractice, losing their licenses, damaging their reputation, or losing confidence when report for errors.

On the contrary, there are positive significance differences between males' and females' respondents for perceived barriers subscale at Fahd hospital except for fear barrier. The reason for this finding may be referred to that most male workforce were foreign (nurses or doctors) who contracted and renew annually so they were afraid from poor professional reputation and financial penalties especially they had family responsibilities which obligate them to maintain contracts.

Furthermore, findings of the present study showed inverse statistical significant differences between doctors and nurses for all perceived barriers subscales at El-Behara hospitals except for administrative barrier. These results are in consistent with several studies which mentioned that incident-reporting behavior differs between medical and nursing professional groups, with nurses reporting significantly more often than doctors[35-38]**.**

Based on a survey among different healthcare professionals, it has been suggested that nurses feel more guilty, worried, embarrassed, and afraid of disciplinary action than doctors and pharmacists as a result of a greater feeling of responsibility for an error, fear of the consequences for the patient and fear of further punishment from senior staff[39]**.**

On the other hand, there are positive statistical significant differences between doctors and nurses for all perceived barriers subscales at King Fahd University Hospital except for burden of effort barrier were inverse without difference. The truth stated that doctors were perceived to report errors less often than nurses. This can be attributed that, physicians believed that nurses were responsible for reporting errors[40]**.** In fact, they not considered more serious events as incidents, but as known complications. However, physicians' willingness to disclose errors may be stimulated by accountability, honesty, trust, and reducing risk of malpractice, physicians may hesitate to disclose because of

professional repercussions, humiliation, guilt, and lack of anonymity[41]. These results are in congruence with a survey of physicians and nurses, physicians identified twice as many barriers to reporting than did nurses both identified time and extra work involved in documenting an error[42].

Results of the present study revealed that no statistical significant differences between two working areas and all perceived barriers' subscales in both settings. These findings are in contradictory with **Mayo and Duncan (2004)**[32] who concluded that nurses working in neonatal ICUs perceived higher reported errors than did those working in medical/surgical units.

In the current study, findings showed highly statistical significant differences between different groups of working hours and perceived barriers subscales at El-Behara hospitals, while the results showed no statistical significant differences at King Fahd University Hospital. In fact, excessive total worked hours puts nurses and physicians at risk; in addition, rotating shifts can also threaten patient safety. Working more than 40 or 50 hours/ week and also increased the odds of making an error[43,44].

Actually, human factors like fatigue are often absent from the analysis of an incident report. Nurses who worked 12 – hours' shifts made more errors in grammatical reasoning and chart reviewing than did nurses who worked shorter shifts. Most reporting systems are complex and time consuming, hence clinicians don't feel they have time to use them. Actually, reporting systems have been relatively cumbersome. The process of completing detailed forms, submitting them up the chain of command, and attending meetings and interviews has deterred many health care professionals from reporting all but the most egregious errors[45]. Moreover, physicians referred to the excessive time required for form filling could be better spent with patients and the menial nature of paperwork that was somehow beneath the medical expertise[43].

Findings of the current study demonstrated highly statistical significant differences between three groups of nationalities and perceived barriers' subscales at King Fahd University Hospital. The highest mean score was for Egyptians, followed by Saudis then foreigners. An explanation for these findings may be due to increasing number of Egyptians' respondents than others, as well as both Egyptians and Saudi had professional responsibility to protect patient safety. Moreover, they were more interested to investigate and explore the causes and barriers for poor reporting system in order to disseminate the results to the administrative body of the hospital and take appropriate actions to enhance reporting system and encourage health care personnel to report errors without fearing of blame and penalties. A recent study showed that language barriers can play a significant role in medical mistakes[46].

In the current study, it can be noticed that respondents in both settings agreed that administrative barrier was the main reason for not reporting error. Indeed a punitive environment to error reporting was perceived as an important feature due to culture of blame within healthcare without organizational leadership and support. Similar studies were supported the previous finding as **Malik et al (2010)**[7], **Chiang and Pepper (2006)**[29] and **Blegen et al (2004)**[30]. A non-supportive environment, a culture of blame and shame and the culture of medicine, with its emphasis on professional autonomy, collegiality, and self-regulation, is unlikely to foster incident reporting[47].

The next strongest perceived barrier was "burden of effort" at El-Behara hospitals and "understanding barrier" at Fahd hospital. At El-Behara hospitals, nurses may be overwhelmed with many roles for providing patient care besides clerk and administrative tasks with no clear job descriptions was available. Moreover, most of doctors are busy and have poor time management skills because they waste a lot of time doing unproductive things. Thus, some doctors will flit from hospital to hospital and travel from one clinic to another, right from one end of the city to another, which means they will often end up spending two or three hours stuck in traffic, just commuting, rather than seeing patients. As a result, both doctors and nurses haven't enough time to fill forms of incident reports. Therefore, error reports are difficult to complete, and feedback about needed system changes to improve safety is not commonly given[48].

At King Fahd University hospital, both doctors and nurses have lack of understanding for what constitute the errors because they shoulder much of the responsibility, job stress and anxiety, lack of appropriate teamwork and effective communication. To improve incident reporting among them, clarification is needed of which incidents should be reported, the process needs to be simplified, and feedback given to reporters.

In one study of clinicians in rural hospitals, the majority agreed that hospital administrators didn't punish error reporters. Most agreed that the hospital culture recognized that mistakes could be made (64%) and that error reporting could be done by all employees (86%). The majority felt comfortable (65%) or somewhat comfortable (32%), discussing medical errors, have learned, and would like to continue to learn from the mistakes of others[49].

Results in the present study demonstrated that the majority of total sample preferred to disclose errors with near misses, followed those who don't prefer to disclose any errors then those preferred to disclose errors with minor harm. Inasmuch as, nurses and physician who wish to act ethically and disclose harm-causing errors are therefore confronted by the possibility of financial and perhaps professional disaster.

Reporting near misses can provide invaluable information for proactively reducing errors. Nonetheless, reporting potentially harmful errors that were intercepted before harm was done, errors that did not cause harm, and near-miss errors is as important as reporting the ones that do harm patients[50]. For minor errors, disclosure may fall to the staff nurse but for more serious ones, it will be the responsibility of the nursing supervisor, department manager or director, nursing executive, executive administration, the physician, or any combination thereof. It depends on what the problem was, who made the error, and the extent of the loss or damage[51].

At El-Behara hospitals, the majority of respondents preferred to disclose errors with near miss and others don't prefer to disclose any errors. An obvious and understandable reason for not disclose major errors is the fear of consequences, such as an angry patient, a complaint sent to the court. Really, physicians and nurses often resist acknowledging offenses and fail to adequately apologize for their mistakes. Similar result reported that both physicians and nurses reported near misses[52].

On the contrary, considerable percent of respondents at King Fahd University Hospital did not prefer to disclose errors and others preferred to disclose errors with minor harm. In fact, the severity of errors and who is doing the reporting influence errors reported. In this respect, one survey found that 58% of nurses didn't report minor medication errors, while another survey suggested that physicians reported more major events and nurses reported more minor events, because they had a more "inclusive view"[53].

Banja (2005)[24] suggested that physician resistant to inform report to maintain a self-image for themselves and others of being strong, always in charge, unemotional, and a perfectionist. The feared loss of this self-image may lead to the unbearable emotion of shame and subsequent feelings of depression. An apology may expose vulnerability, remove emotional armor, and allow emotions to be exposed. Medical professionals and colleagues need to work at tolerating and supporting their own humanity and that of their colleagues.

On concerning personal preference to disclose errors for patients or their families, results showed that the majorities of respondents did not prefer to disclose errors for them, and also did not prefer to report errors for college, head nurse, or chief executive officer in both settings. These findings ought not be surprising due to fear of litigation, fear of losing the support of their colleagues or the organization in which they practice, fear from losing their professional and personal reputation, strong cultural reluctance within medicine and nursing to admit mistakes.

It is no accident that 45.6% of respondents preferred to report errors for physicians at El-Behara hospitals and 67.7% of respondents preferred to report errors for supervisors at Fahd hospitals. Thus one possible explanation for this finding is that in Egypt more friendship and support were established between nurses and doctors, while in KSA more restrictive and complicated hierarchy must be followed with limitation for friendship among staff.

Furthermore , the literature suggests other health professionals beyond physicians fail to disclose errors for several reasons including, a desire to protect patients and their family from any additional anxiety and distress, to prevent undermining the patients trust in the care that they are receiving and to protect their relationship with the patient and their family[54-56].

Based upon our findings, there are highly statistical significant correlations between all subscales of perceived barriers at El-Behara hospitals, while at King Fahd University Hospital the correlations of fear with administrative and burden of work are not statistically significant. Both physicians and nurses in two settings view the perceived barriers as a significant barrier to error reporting.

Conclusions

Sustained and collaborative efforts to reduce the occurrence and severity of health care errors are required so that safer, higher quality care results. To improve safety, error- reporting strategies should include identifying errors, admitting mistakes, correcting unsafe conditions, and reporting system.

The present study demonstrated underreporting of adverse events by both nurses and physicians due to administrative barrier which considered as a major barrier. The next strongest perceived barrier was burden of effort at El-Behara hospitals and understanding barrier at King Fahd University Hospital. The total sample preferred to disclose errors with near miss, followed those who don't prefer to disclose any errors then those preferred to disclose errors with minor harm. Furthermore, the majorities of respondents did not prefer to inform errors for patient, college, head nurse or chief executive officer.

While some of respondents preferred to report errors for physician at El-Behara and for supervisor at King Fahd University Hospitals.

Recommendations

The results of current study indicated the need to improve the accuracy of error reporting by nurses and physicians to provide a hospital environment conducive to preventing errors from occurring. Hence, healthcare professionals should receive education, training, and practice in disclosure, and health care institutes should establish non-punitive policies of error reporting and implement full disclosure policies. Hence, successful plans to support physicians and nurses will necessarily start with leadership at the top of organizations.

Institutions should also ensure that error reporting systems are confidential, simple, and worthwhile. To convince physicians and nurses that reporting errors is not a fruitless exercise, institutions should advertise examples that display the connection between error analysis and system improvement. Institutions should also teach physicians and nurses how to report errors and what errors to report. Finally, a disclosure team should be formed including associated staff and management along with risk management personnel and/or the organization's legal counsel, at least in the planning stage. Future study is required to further investigate these findings and improve reporting rates.

Acknowledgments

The authors would like to acknowledge the invaluable assistance of Assistant Lecturer/ Heba Obied and my master's student/ Rehab Mary for their helping during the period of data collection.

References

1. White A. A., McCotter P., Boyle D. J., M. D. and Gallagher T. H. Supporting Health Care Workers After Medical Error: Considerations for Health Care Leaders. JCOM. 2008; 15(5): 240-7.
2. Boyle D., O'Connell D., Platt F. W. and Albert R. K. Disclosing Errors and adverse events in the intensive care unit. J of Crit Care Med. 2006; 34(5); 1532- 1537.
3. Kingston M. J., Evans S. M., Smith B. J. and Berry J. G. Attitudes of Doctors and Nurses towards Incident Reporting: A Qualitative Analysis. MJA. 2004; 181 (1): 36-39.
4. Terzibanjana A. R., Laaksonenb R., Weissb M., Airaksinena M., Wulijic T. Medication Error Reporting System– Lessons Learnt. International Pharmaceutical Federation (FIP). Netherlands: 2007; 1-7.
5. Koohestani H. R. and Baghcheghi N. Barriers to the Reporting of Medication Administration Errors among Nursing Students. Australian Journal of Advanced Nursing. 2009; 27(1): 66-74.
6. LeRoy L. and Treanor K. M. Patient Safety: Grant Makers Join The Effort To Reduce Medical Errors. Health Affairs. 2001; 20(2):287-290.
7. Malik M. R., Alam A.Y., Mir A. S., Malik G. M. and Abbas S. M. Attitudes and Perceived Barriers of tertiary Level Health Professionals Towards Incident Reporting in Pakistan. North American J of Medical Sciences. 2010; 2(2): 100-5.
8. Scott L. D., Rogers A. E., Hwang W. T. and Zhang Y. Effects of Critical Care Nurses' Work Hours on Vigilance and Patients' Safety. Am J Crit Care. 2006; 15: 30-37.
9. Hughes R. G. and Ortiz E. Medication errors: Why they Happen, and How They can be Prevented. American J Nurs. 2005; 105(3): 14-20.
10. Steven M. and Selbst M. D. The Difficult Duty of Disclosing Medical Errors. Contemporary Pediatrics Journal. 2003; 1-8.
11. Hammami M. M., Attalah S. & Al Qadire M. Which Medical Error to Disclose to Patients and by Whom? Public Preference and Perceptions of Norm and Current Practice. BMC Medical Ethics Journal. 2010; 11-17.
12. Addressing Health Care Errors under Medicare Report to the Congress: Selected Medicare Issues, 1999; Ch 3.
13. ABIM Foundation, American Board of Internal Medicine, ACP-ASIM Foundation, American College of Physicians-American Society of Internal Medicine, European Federation of Internal Medicine. Medical Professionalism in the New Millennium: a Physician Charter. Ann Intern Med. 2002; 6; 136(3):243-246.
14. Lawton R. and Parker D. Barriers to Incident Reporting in a Health Care System. Qual Saf Health Care. 2002; 11:15-8.
15. Runciman W., Merry A. and Smith AM. Improving Patients' Safety by Gathering Information. Anonymous reporting has an Important Role. BMJ 2001; 323:7308.
16. Serembus J. F., Wolf Z. R., Youngblood N. Consequences of Fatal Medication Errors for Health Care Providers: A Secondary Analysis Study. MEDSURG Nurs. 2001; 10(4):193-201.
17. Wolf Z. R., Serembus J. F., Smetzer J., Cohen H., and Cohen M. Responses and Concerns of Health Care Providers to Medication Errors. Clin Nurs Spec. 2000; 14(6):278-89.
18. Riley W., Davis S., Miller K., Sweet R. and Hansen H. Improved Patient Safety: Near Misses, Errors and Breaches. International Conference of Healthcare Systems Ergonomics and Patient Safety 2008, Strasbourg, France, 2008.
19. Boyle D., O'Connell D., Platt F. W. and Albert R. K. Disclosing Errors and Adverse Events in the Intensive Care Unit. Crit Care Med. 2006; 34 (5):1532-1537.
20. Nosek R. A., McMeekin J., Rake G. W. Standardizing Medication Error Event Reporting in the U.S. Department of Defense. J of Advances in Patient Safety. 2005; Vol. 4; 361-74.
21. Edwin A. K. Non-Disclosure of Medical Errors an Egregious Violation of Ethical Principles. Ghana Medical J. 2009; 43(1): 34-39.
22. Devers K. J., Pham H. H. and Liu G. What is Driving Hospitals' Patient-Safety Efforts? J Health Affairs. 2004; 23(2):103-115.
23. Cook A. F., Hoas H. Handbook for Rural Health Care Ethics: a Practical Guide for Professionals. University Press of New England. 2009; Chapter 12, 234-7.
24. Banja J. D. Does Medical Error Disclosure Violate the Medical Malpractice Insurance Cooperation Clause? J of Advances in Patient Safety. 2005; 3: 371-82.
25. ECRI Institute. Risk and Quality Management Strategies 21: Culture of safety. 2009, Accessed at:

http://www.ecri.org/documents/patient_safety_center/hrc_cultureof Safety.pdf.
26. Rapala K. Mentoring Staff Members as Patient Safety Leaders: the Clarian Safe Passage Program. J of Crit Care Nurs Clin North Amer. 2005; 17:121-6.
27. Wakefield B., Wakefield D. S. & Uden-Holman T. Improving Medication Administration Error Reporting Systems. Ambulatory Outreach; 2000; 16-20.
28. Joolaaee S. F., Hajibabaee H., Peyrovi H., Haghani H., and Bahrani N. The Incidence and Report Rate of Medication Errors in Iran. J. Med. Ethics History Med. 2010; 3(1): 65-76.
29. Chiang H. Y., Pepper G. A. Barriers to Nurses' Reporting of Medication Administration Errors in Taiwan. J Nurs Scholarship. 2006; 38(4):392-399.
30. Blegen M. A., Vaughn, T., Pepper G., Vojir, C., Stratton K., and Boyd M. Patient and Staff Safety: Voluntary Reporting. American J of Medical Quality. 2004; 19(2): 67–74.
31. Evans S. M., Berry J. G., Smith B. J., Esterman A., Selim P., O'Shaughnessy J., and DeWit M.. Attitudes and Barriers to Incident Reporting: a Collaborative Hospital Study. Qual Saf Health Care 2006; 15:39-43.
32. Mayo A. M. and Duncan D. Perceptions of Medication Errors What We Need to Know for Patient Safety. J Nurs Care Qual. 2004; 19(3): 209–217.
33. Kim J., An K., Kim M. K., Yoon S. H. Nurses' Perception of Error Reporting and Patient Safety Culture in Korea. West J Nurs Res Online 2007; 1-18.
34. Vojir C. P., Blegen M. A., Vaughn T. Nursing Staff as Estimators of Unit Medication Error Reporting. Commun Nurs Res. 2003; 36:202.
35. Evans S. M., Smith B. J., Esterman A., Runciman W. B., Maddern G., Stead K. Selim P., O'Shaughnessy J. Muecke S. and Jones S. Evaluation of an Intervention Aimed at Improving Voluntary Incident Reporting in Hospitals. Qual Saf Health Care 2007; 16:169
36. Hirose M., Regenbogen S. E., Lipsitz S., et al. Lap Time in an Incident Reporting System at a University Hospital in Japan. Qual Saf Health Care. 2007; 16:101-4.
37. Harris C. B., Krauss M.J., Coopersmith C. M., Avidan M., Nast P. A., Kollef M. H., Dunagan W. C., and Fraser V. J. Patient Safety Event Reporting in Critical Care: a Study of Three Intensive Care Units. Crit Care Med. 2007; 35(4):1068-76.
38. Osmon S., Harris C. B., Dunagan W. C., Prentice D., Fraser V. J. and Kollef M. H. Reporting of Medical Errors: an Intensive Care Unit Experience. Crit Care Med 2004; 32:727-33.
39. Sarvadikar A., Prescott G. and Williams D. Attitudes to Reporting Medication Error among Differing Healthcare Professionals. Eur J Clin Pharmacol. 2010; 6(8):843-53.
40. Harper M. L. and Helmreich R. L. Identifying Barriers to the Success of a reporting system. In: Henriksen K., Battles J. B., Marks E. S., and Lewin D. I. Advances in Patient Safety: from Research to Implementation. Agency for Healthcare Research and Quality. AHRQ Publication No. 05-0021-3, 2005;167-79
41. Kaldjian L. C., Jones E. W., Rosenthal G. E., Tripp-Reimer T., and Hillis S. L. An Empirically Derived Taxonomy of Factors Affecting Physicians' Willingness to Disclose Medical Errors. J Gen Intern Med. 2006; 21:942-8.
42. Uribe C. L., Schweikhart S. B., Pathak D. S., Dow M. and Marsh G. B. Perceived Barriers to Medical-Error Reporting: an Exploratory Investigation. J Health Care Manag. 2002; 47(4):263-80.
43. Scott L.D., Roger A.E., Hawang W. and Zhang Y. Effects of Critical Care Nurses' Work Hours on Vigilance and Patients' Safety. American Journal of Critical Care. 2006; 15(1): 30-7.
44. Rogers A. E., Hwang W. T., Scott L. D., Aiken L. H., and Dinges D. F. The Working Hours of Hospital Staff Nurses and Patient Safety. 2004; Health Affairs. *2 3(4): 202-12.*
45. Twerski R. A. J. Medical Errors: Focusing More on What and Why, Less on Who. J of Oncology Practice. 2007; 3 ;(2) : 66-70.
46. Medical College of Wisconsin. Language Barriers Lead to Medical Mistakes available at http://healthlink.mcw.edu/article/1031002276.html. 2003.
47. Barach P., Small S. D. Reporting and Preventing Medical mishaps: Lessons from Non-Medical Near-Miss Reporting System. BMJ. 2000; 320:759-63.
48. Woods D. and Cook R. From Counting Failures to Anticipating Risks: Possible Futures for Patient Safety. In Zipperer L, Cushman S. Lessons in Patient Safety: A Primer. National Patient Safety Foundation; Chicago, IL; 2001.
49. Cook A. F., Hoas H., Guttmannova K. and Joyner J. C. An Error by Any Other Name. Am J Nurs 2004; 103:32-43.
50. Moskop J. C., Geiderman J. M., Hobgood C. D., and Larkin G. L. Emergency Physicians and Disclosure of Medical Errors. American College of Emergency Physicians. 2006; 48(5): 523-31.
51. Kaldjian L. C., Jones E. W. , Rosenthal G. E., Tripp-Reimer T. and Hillis S. L., An Empirically Derived Taxonomy of Factors Affecting physicians' Willingness to Disclose Medical Errors. J Gen Intern Med. 2006; 21:942-8.
52. Day S, Fox J, Reading T. Adverse Event Reporting: Experience and Education of Trauma Personnel at a Level One Trauma Center. J Trauma Nurs. 2004; 11(4):137-43.
53. Osborne J, Blais K, Hayes JS. Nurses' Perceptions: When Is It a Medication Error? J Nurs Adm. 1999; 29(4):33-8.
54. Gallagher T. H., Studdert D. and Levinson W. Disclosing Medical errors to patients. The New England Journal of Medicin. 2007; 28: 2713-19.
55. Gallagher T. H., Garbutt J. M., Waterman A. D., Flum D. R., Larson E. B., Waterman B. M., Dunagan W. C., Fraser V. J. and Levinson W. Choosing Your Words Carefully. How Physicians Would Disclose Harmful Medical Errors to Patients. 2006; 166(14): 1585-93.
56. Sorensen R., Iedema R., Piper D., Manias E., Williams A., Tuckett A. Disclosing Clinical Adverse Events to Patients: Can Practice Inform Policy? Health Expect. 2009; 13(2): 148-59.

Mice (*Mus musculus*) genome responses to methotrexate (MTX) and some plant extracts

Mansour, A.A[1,2]. M.A. Salam[1], and Y. M. Saad[3,4]

[1]Dept. of Genetics, Faculty of Agric., Ain Shams Univ., Shobra El-Kheima, Egypt.
[2]Dept. of Biotechnology, Faculty of Applied Medical Sciences, Taif University, Trubah, KSA
[3]Dept. of Biol. Science, Fac. of Sciences, King Abdulaziz Univ., KSA.
[4]Genetic Lab., National Institute of Oceanography and Fisheries (NIOF), Egypt.
yasser_saad19@yahoo.com

Abstract: Mice genome responses to MTX (anticancer drug) and some plant (Curcuma, Ginger, Green Tee and Pomegranate) extracts were studied. Mice were intraperitoneally injected with 0.75 mg/kg chronic dose of MTX for two weeks. Mortality percentages, bone marrow cell divisions, morphological and biochemical characterization of treated mice groups were estimated. The mortality percentage was 40 % in the positive control while no mortality was observed in both (MTX, Curcumin) and (MTX, Pomegranate) treatments. Some treated mice had ulceration and hair loss on the ears skin. No morphological changes were observed on the negative control group. At cytogenetic level, no bone marrow cell divisions were detected in treated mice. Two Isozyme systems (Esterase and Super oxide dismutase) and Protein electrophoresis were assayed to detect biochemical genetic markers for all mice groups. These analyses reflect mice genome responses under experimental conditions.
[Mansour, A.A. M.A. Salam, and Y. M. Saad. **Mice (*Mus musculus*) genome responses to methotrexate (MTX) and some plant extracts.** *Life Sci J* 2012;9(4):4881-4886] (ISSN:1097-8135). http://www.lifesciencesite.com.733

Keywords: *Mus musculus*, Methotrexate, SDS, Isozyme, Morphology.

1. Introduction

Methotrexate (MTX, Amethopterin) chemotherapy drug is a cell cycle-specific analog, active in S-phase of the cell cycle, inters cells through specific transport system mediated by the reduced folate carrier and the folate receptor protein. It has a cytotoxic effect with an anti-folate metabolite that inhibit folate-dependent enzymes such as *thymidylate synthase*, which further blocks the de novo purine synthesis by directly inhibiting the activity of *5-aminooimidazole-4-carboxamide ribonucleotide transformylase*, causing an increase in both intracellular and extracellular adenosine (an potent anti-inflammatory mediator) (Cronstein, 2005; Cutolo *et al.*, 2001), and an increase in cAMP (Cutolo *et al.*, 2001), thus blocking the process of DNA synthesis and arrests the cell proliferation. MTX is widely used in treatments of different type of cancers, rheumatoid arthritis (RA) and other chronic inflammatory conditions (Minaur *et al.*, 2002).

MTX like other anticancer drugs has a lot of painful side effects like hair losing, ulcerations, emetic, decreases in attention/concentration, speed of information processing and memory and failure of liver and/or kidney functions (Brezden *et al.*, 2000, Schagen *et al*., 2002, Kreukels *et al.*, 2005, Shilling *et al.*, 2005 and Winocur *et al.* 2006). Up to date, there are no conventional treatments were applied to avoid the MTX side effects on patient. So, unconventional treatments should be applied to avoid side effects of MTX and other drugs (Eisenberg *et al.*, 1993, Murray *et al.*, 1992, and Visser *et al.,* 1992).

Curcuma (*Curcuma longa*), Ginger (*Zingiber officinale*), green tea (*Camellia sinensis*) and pomegranate (*Punica granatum*) plants are rich in different anti-oxidants, anti-inflammatory, and anti-cancer components (Venkatesan *et al.*, 2000, Antunes *et al.*, 2001, Ammon and Wahl 1991, Lin 2007, Srivastava and Mustafa 1992, Sharma *et al.* 1994, Afzal *et al.*, 2001, Rogers *et al.* 1998, Yang *et al.* 1998, and Isemura *et al.* 2000, Mayer *et al.*, 1977; Tanaka *et al.*, 1986a, Du *et al.*, 1975, Lansky *et al.*, 1998, Schubert *et al.*, 1999, Mayer *et al.*, 1977; Tanaka *et al.*, 1986a and Du *et al.*, 1975, Lansky *et al.*, 1998, Schubert *et al.*, 1999). So, the extracts of these plants were chosen as unconventional treatments to test its ability to face the side effects of MTX treatments.

The main aims of this work:

1- Investigation of the mice genome responses to methotrexate (MTX) and some plant extracts (Curcumin, Ginger, Pomegranate fruit and green Tea) as unconventional treatments.
2- Detection the effects of these treatments on the mice morphological characterization.

2. Material and Methods

Mice (*Mus musculus*) animals:

Healthy and genetically pure mice males (20 ± 5g) were housed for one week before experiments for acclimatization to the laboratory conditions.

Treatment with Methotrexate (MTX):

MTX treatments and LD_{50} determination were carried out as described by Pelker *et al.*, (1985) and Wheeler *et al.*, (1995) with some modifications. MTX were intra peritoneal injected (0.75 mg/kg) as chronic doses. The Treatments were divided into six groups (ten male mice in each). Treatment design was presented in Table (1):

Table (1): Mice groups and description of treatments.

Group	Description
Negative control (C-)	Drank water without methotrexate (MTX) injection
Positive control (C+)	Injected with MTX drug once day after day for 2 weeks with a 0.75 mg/kg (Pelker *et al.*, 1985; Wheeler *et al.*, 1995), a dosage similar to clinical therapeutic usage (Friedlaender, *et al.*, 1984).
T1	Intraperitoneally injected with MTX and drank socked Curcumin instead of water.
T2	Intraperitoneally injected with MTX and drank socked Ginger powder instead of water.
T3	Intraperitoneally injected with MTX and drank Pomegranate juice instead of water.
T4	Intraperitoneally injected with MTX and drank socked green Tea instead of water.

Plants material:

Curcumin or Cr (*Curcuma longa*), Ginger or Gn (*Zingiber officinale*), green tea or Te (*Camellia sinensis*) and pomegranate or Pg (*Punica granatum*) plants obtained from Faculty of Agric., Ain Shams Univ., Shobra El-Kheima, Egypt.

Ten grams of each Curcumin, ginger powder and green Tea were soaked separately overnight in 1 litter of hot water (60^oC). On the other hand pomegranate fruit (200g) was blinded in 1 litter of fresh water and used directly for mice drinking instead of water for one week before injection with MTX, except negative and positive controls.

Bone marrow preparation and cytogenetical analysis:

Two hours before sacrificing, all mice were intraperitoneally injected with 0.6 mg\kg colchicine (Sharma and Sharma, 1994). The bone marrow cells were collected from mice femurs using a 0.075 M KCl. Centrifuged for 5 min at 1500 rpm, re-suspended in 0.075 M KCl and incubated at 37^oC for 15 min. After fixation, Bone marrow cells were dropped onto slides (previously stored at 4^oC in cold 70% ethanol) and stained 10% Giemsa (Merk) (Boxio, *et al.*, 2004).

Biochemical genetic analysis:

Protein extraction:

The mice lever (0.5g) samples were obtained from each applied animal and extracted in appropriate volume of 0.85% NaCl solution (1ml). The extraction was homogenized then centrifuged at 12000 rpm/10min. The supernatants were applied for electrophoresis.

Protein separation:

25µl from each protein supernatant was diluted with an equal volume of 2X SDS Leans buffer according to Laemmli (1970) and modified by Abdel-Reheem *et al*., (2007). Samples were applied to 15% polyacrylamid gel. Gel preparation, electrophoresis conditions, staining and destaining gels were done as described by Abdel-Reheem *et al*., (2007) with some modifications.

Isozyme systems:

Esterase (Est.) with different substrates (α-naphthylacetate, α-naphthylvalerate) and Super oxide dismutase (SOD) Isozyme systems were applied to discriminate biochemical variations among treated and control samples. Electrophoretic conditions, gel preparation, staining and distaining were carried out according to Tanksley and Rick (1980), Tanksley and Orton (1983) and Saad (2002).

3. Results

Effects of different Treatments on mice morphological characterization:

The effects of different Treatments with Methotrexate (MTX) alone or doubled with soaked Curcumin (Cr) or Ginger (Gn), green Tea (Te) or Pomegranate juice (Pg) were presented in Table (2).

Table (2): Remarked characters correlated with Methotrexate and plant materials treatments.

Case	C-	C+	Cr	Gn	Pg	Te
Injections (MTX)	0	5	5	5	5	5
Ear Ulceration	-/-	+/+	-/-	+/+	-/+	+/+
Mice Activity	high	low	high	moderate	high	moderate

(+) means ulceration for one ear. (-) means normal ear

The negative control (C-) group exhibit normal (morphology and performance). Ear ulceration was a good remarkable observation of the treated mice. Both ears of treated mice were ulcerated in the positive control (treated only with MTX) as presented in figure (1a). Four mice had ulceration in one/both ears in the group of (MTX, Gn) double treatment (Figure 1b). Five mice of (MTX, Te) group had ulceration in both ears (Figure1c). In the case of MTX with Cr both ears of all mice were normal without any ulceration as shown in figure (1d) comparing with the negative control (figure 1e). While in MTX, Pg treatment, three mice had red spots (beginning of ulceration formation). Only one ear with red spots was noted as shown in figure (1f).

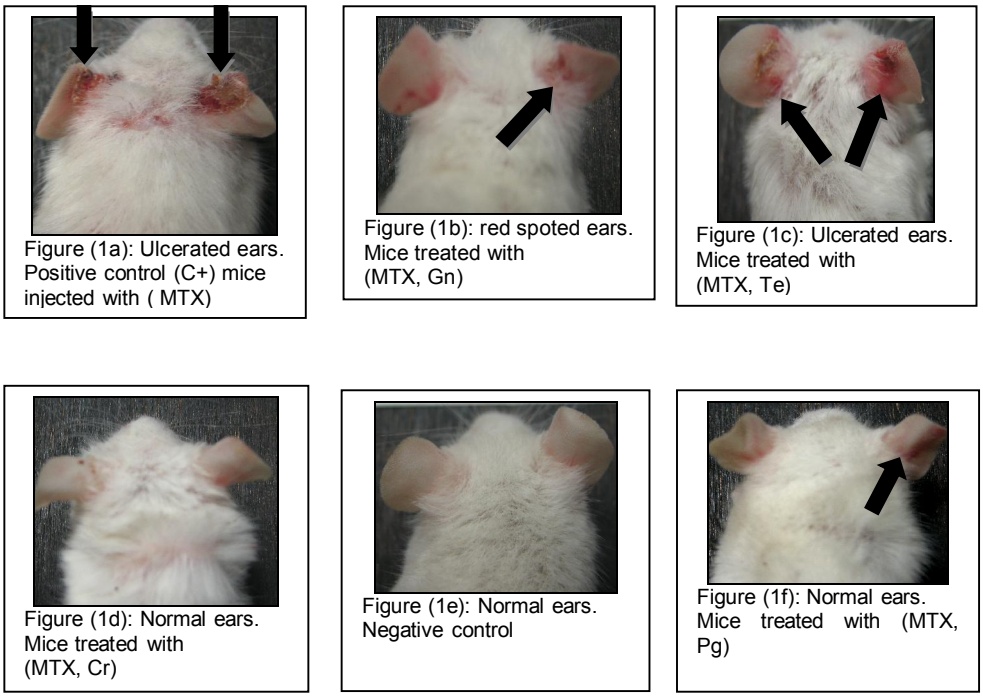


Figure (1a): Ulcerated ears. Positive control (C+) mice injected with (MTX)

Figure (1b): red spoted ears. Mice treated with (MTX, Gn)

Figure (1c): Ulcerated ears. Mice treated with (MTX, Te)

Figure (1d): Normal ears. Mice treated with (MTX, Cr)

Figure (1e): Normal ears. Negative control

Figure (1f): Normal ears. Mice treated with (MTX, Pg)

Figure (1): Morphological characterization of applied mice animals under experiment conditions. (C-) =Negative control, (C+) = Positive Control, (MTX) = methotrexate, (Cr) = Curcumin, (Gn) = Ginger, (Te) =green Tea and (Pg) = Pomegranate

Mortality levels under experiment conditions:

The percentage of mortality was 40% in the positive control (C+) group, (after five MTX injections). The mortality percentage was about (20%) in both (MTX, Gn) and (MTX, Te) treatments. On the other hand, no mortality was noted in both in the (MTX, Cr) and (MTX, Pg) treatments (Figure 2).

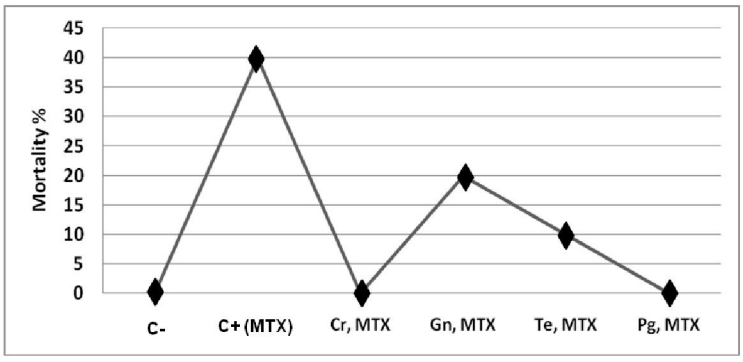


Figure (2): The experiment mortality percentages. (C-) = Negative control, (C+) = Positive Control, (MTX) = methotrexate, (Cr) = Curcumin, (Gn) = Ginger, (Te) = green Tea and (Pg) Pomegranate.

Effects of different Treatments on mice bone marrow cell divisions:

No spread chromosomes were detected in all MTX treatments, only compact nucleuses (undivided) were observed. Negative control showed normal spread chromosomes.

Biochemical genetic analysis:

SDS-PAGE and two isozyme systems (SOD and Est. with two different substrates) were applied to study the effects of different treatments on some liver proteins.

The effects of different treatments on protein electrophoresis:

Protein pattern (figure 3a) showed an extra band in negative control at about 190 kD which disappeared in positive control and other double treatments. It means that, all double treatments (which contain plant extraction) could not prevent MTX effects. In addition, protein banding pattern of the positive control had an extra 2 bands at 40 KD and 35 KD (duo to MTX effects). These 2 bands were absent in negative control and double treatments. Whereas a common band at 34 KD was detected in negative control and double treatments (absent in positive control). One band (200 KD) was detected in double treatment and positive control which is disappeared in the negative control. This band is considered as marker for the effect of MTX and the interaction between MTX and plant extracts on liver gene expressions.

The effects of different treatments on isozyme electrophoresis:

The electrophoresis pattern of SOD isozyme showed that, one band at RF=0.38 was detected in positive control and all other treatments while absent in negative control. This illustrate that this band is created under MTX effect (figure 3b).

The electrophoresis pattern of Est. with α-naphthyl acetate isozyme (Figure 3c) showed that, One band at RF= 0.12 was appeared in T1 (Cr, MTX) and not appeared in the others (C-, C+, T2, T3 and T4).

Esterase (Est.) with α-naphthyl vallerate:

Regarding Esterase (Est.) with α-naphthyl vallerate pattern (figure 3d), only one band with RF about 0.04 was appeared in the treatments (C-, C+, T2, T3 and T4) and absent in (T1).

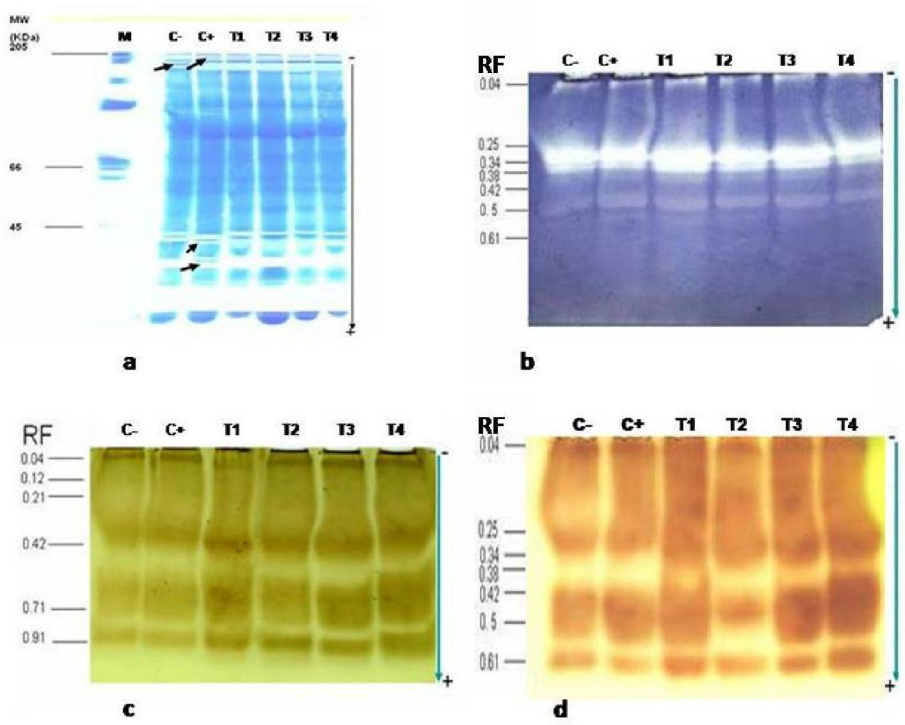


Figure (3): SDS PAGE Protein (a), SOD (b), Est. *α*-naphthyl acetate (c) and Est. *α*-naphthyl vallerate banding patterns. MW = Molecular weight (KD), RF = relative front, C- = Negative control, C+ = Positive Control, and T = Treatment.

4. Discussion

Mice were injected with MTX dosage similar to clinical therapeutic usage (Friedlaender, *et al.*, 1984). The mortality ratio was 40 % in the positive control. Whereas in the double treatment (MTX, Cr) no mortality was observed as well as (MTX, Pg) treatment, these results reflect that Curcumin and pomegranate decreases the toxicity effect of MTX and prevent appearance of ulceration on mice ears (as a secondary symptoms appeared in the positive control). These results agree with the explanation of Venkatesan *et al.*, 2000 and Antunes *et al.*, 2001 that Curcumin prevents or attenuates nephrotoxicity caused by adriamycine or cisplatin chemotherapy treatments respectively. The effects of pomegranate juice may be referred to its enrichment with phenolic compounds and its antioxidant activity (Schubert *et al.*, 1999). In the case of MTX, ginger and MTX, green tea, the viability was near to be same but the ulceration on mice ears was varied, it was only red spots appeared in MTX, ginger treated mice, while it was sever in the case of MTX, Tea treatment. The lower mortality appeared in both cases may be referred to the phenolic compounds of ginger and green Tea (Lee and, Surh, 1998; Wang *et al.*, 2003; Chung *et al.*, 2001) and (Komori *et al.* 1993) respectively.

Methotrexate acts as folate analog. It has an inhibition effect on dihydrofolate reductase (DHFR) resulting in depletion of critical reduced folates, thymidylate synthesis as well as purine synthesis, and incorporates a dUTP bases into DNA resulting in inhibition of DNA synthesis and function, finally arresting cell division in S-phase (Edward and Vincent 2008), for that, there were not any spread chromosomes observed in bone marrow of all treatments compared with untreated negative control, these results can be explained depending on the efficacy of MTX on bone marrow. These also mean that, Curcumin, ginger, green tea, and pomegranate treatments did not affect the main effect of methotrexate (arresting cell division in S-phase). Georgiou *et al.*, 2009 found that acute MTX chemotherapy transiently depletes the bone marrow of its steady-state haematopoietic and stromal progenitors, reducing haematopoietic cellularity and osteogenesis and increasing marrow fat content, which have the capacity to recover by day 14 subsequent to damage.

Severe bone marrow damage is a known adverse effect of cancer chemotherapy. Xian *et al.,* 2007 employed a rat model of an acute Methotrexate (MTX) chemotherapy and demonstrated that daily methotrexate injections at 0.75 mg/kg for five consecutive days caused decreased trabecular bone in tibia as well as an increased adipocyte (AD) density in the bone marrow on day 9 post-treatment.

Several techniques have been used, with different degrees of success, to identify and trace minor differences among animal populations and detect the genetic responses of any treatments. These include morphometries, karyotype analysis, serum protein analysis, immunology and agglutination assay, isozyme polymorphism and protein banding patterns. Among these techniques, however, biochemical studies of genome responses for treatments of populations through electrophoretic analysis are advantageous (Saad *et al*. 2002).

References:

1. AbdeL-Reheem, M. A., Y. M. Saad, M. A. Sallam and T. M. A. Tantawy (2007).Responses of some Tilapia genomes to oleic acid. Journal of biological chemistry and environmental sciences. 2 (4). 407 - 430.
2. Afzal M, Al-Hudidi D, Menon M, Pesek J, Dhami MS. (2001): Ginger: an ethnomedical, chemical and pharmacological review. Drug Metabol Drug Interact; 18: 159 – 90.
3. Ammon H. P. and M. A. Wahl (1991): Pharmacology of Curcuma longa Planta Med. 57: 1 – 7.
4. Antunes, L.M., J.D. Darin, N. Bianchi and L. de (2001): Effects of the antioxidants curcumin or selenium on cisplatin-induced nephrotoxicity and lipid peroxidation in rats. Pharmacol. Res. 43, 145 – 150.
5. Boxio R., Carine Bossenmeyer-Pourie´, Natacha Steinckwich, Christian Dournon, and O. Nuesse (2004): Mouse bone marrow contains large numbers of functionally competent neutrophils. *J. Leukocyte Biology* 75, 1-8.
6. Brezden C. B., K. A. Phillips, M. Abdolell, T. Bunston, and I. F. Tannock (2000): Cognitive function in breast cancer patients receiving adjuvant chemotherapy. J. Clin. Oncol. 14: 2695 – 701.
7. Chung W. Y., Y. J. Jung, Y. J.Surh, S. S. Lee, and K. K. Park (2001): Antioxidative and antitumor promoting effects of [6]-paradol and its homologs. *Mutat. Res.* 496: 199 – 206.
8. Cronstein B. N. (2005): Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis. Pharmacol Rev.; 57: 163 – 72.
9. Cutolo M., A. Sulli, C. Pizzorni, B. Seriolo, R. H. Straub (2001): Anti-inflammatory mechanisms of methotrexate in rheumatoid arthritis. Ann. Rheum. Dis. 60:729 – 35.
10. Du, C. T.; Wang, P. L.; Francis, F. J. Anthocyanins of pomegranate, *Punica granatum*. *J. Food Sci.* 1975, *40*, 417- 418.
11. Edward Chu and Vincent T. DeVita Jr. (2008): Physicians' Cancer Chemotherapy Drug Manual. Jones and bartett publishers, Sudbury Massachusetts, 259-264.
12. Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL. Unconventional medicine in the United States. Prevalence, costs, and patterns of use. *N Engl J Med*. 1993; 328: 246 – 252.
13. Friedlaender G.E., R.B. Tross, A.C. Doganis, J.M. Kirkwood and R. Baron (1984): Effects of chemotherapeutic agents on bone: short-term methotrexate and dororubicin treatment in a rat model. *J. Bone J. t. Surg.*; 66A: 602 – 7.

14. Georgioua K.R., M.A. Schererb, C.J. Xian and B.K. Foster (2009): Damage and recovery of bone marrow microenvironment after acute Methotrexate chemotherapy j.bone. Vol.: 44, Supplement 1, 163
15. Isemura M., K. Saeki, T. Kimura, S. Hayakawa, T. Minami and M. Sazuka (2000): Tea catechins and related polyphenols as anticancer agents. *Biofactors* 13, 81-85.
16. Komori A., J. Yatsunami, S. Okabe A.L. ET (1993): Anticarcinogenic activity of green tea polyphenols. *Jap J. Clin. Oncol*. 23, 186-190.
17. Kreukels B.P., S.B. Schagen, K.R. Ridderinkhof, W. Boogerd, H.L. Hamburger, F.S. van Dam (2005): Electrophysiological correlates of information processing in breastcancer patients treated with adjuvant chemotherapy. *Breast Cancer Res Treat*; 1: 53 – 61.
18. Laemmli, U.k. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. (227):680-258.
19. Lansky, E.; S. Shubert, and I. Neeman, (1998): Pharmacological and therapeutical properties of pomegranate. In *Proceedings 1st International Symposium on Pomegranate*; Megarejo, P.; Martı´nez, J. J.; Martı´nez, J., Eds.; CIHEAM, Orihuela, Spain,; Pr-07.
20. Lee E. and Y.J. Surh (1998): Induction of apoptosis in HL-60 cells by pungent vanilloids, [6]-gingerol and [6]-paradol. *Cancer Lett;* 134:163–8.
21. Lin JK. Molecular targets of curcumin. Adv Exp Med Biol 2007; 595: 227–43.
22. Mayer, W.; A. Go rner and K. Andra (1977): Punicalagin und punicalin, zwei gerbstoffe aus den schalen der granata pfel Liebigs. *Ann. Chem*., 1976-1986.
23. Minaur NJ, Kounali D, Vedi S, Compston JE, Beresford JN, Bhalla AK. Methotrexate in the treatment of rheumatoid arthritis. II. In vivo effects on bone mineral density. Rheumatology (Oxford) 2002;41:714–49.
24. Murray RH, Rubel AJ. Physicians and healers-unwitting partners in health-care. *N Engl J Med*. 1992; 326 (suppl 1): 61 – 64.
25. Pelker R. R, G. E, Friedlaender, M. M. Panjabi, T. Markham, M. Hausman, A. C. Doganis, *et al.* (1985): Chemotherapy-induced alterations in the biomechanics of rat bone. *J Orthop Res.* 3:91–5.
26. Rogers A.E., L.J. Hafer, Y.S. Iskander and S. Yang (1998): Black tea and mammary gland carcinogenesis by 7, 12- dimethylbenz [a] anthracene in rats fed control or high fat diets. Carcinogenesis 19, 1269-1273.
27. Saad T. M. (2002): Genetic fingerprint of some *Oreochromis lines*. M. Sc. Ain Shams University.
28. Schagen SB, Muller MJ, BoogerdW, Rosenbrand RM, van RD, Rodenhuis S, *et al*. (2002): Late effects of adjuvant chemotherapy on cognitive function: a follow-up study in breast cancer patients. Ann Oncol;9: 1387–97.
29. Sharma, A. K. and A. Sharma (1994): Chromosome Techniques: A manual. Harwood Academic Publishers, Switzerland.
30. Sharma J.N., K.C. Srivastava, E.K. Gan (1994): Suppressive effects of eugenol and ginger oil on arthritic rats. *Pharmacology*. 49: 314 – 318.
31. Shilling V, Jenkins V, Morris R, Deutsch G, Bloomfield D. The effects of adjuvant chemotherapy on cognition in women with breast cancer—preliminary results of an observational longitudinal study. Breast 2005;2:142–50.
32. Schubert, S. Y.; E. P. Lansky and I. Neeman, (1999): Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. *J. Ethnopharmacol*., *66*, 11-17.
33. Srivastava K.C., and T. Mustafa (1992): Ginger (*Zingiber officinale*) in rheumatism and musculoskeletal disorders. *Med Hypotheses*.; 39: 342 – 348.
34. Tanaka, T.; G. I. Nonaka and I. Nishioka (1986a): Tannins and related compounds. XL. Revision of the structures of Punicalin and Punicalagin, and isolation and characterization of 2-Galloylpunicalin from the bark of *Punica granatum* L. *Chem. Pharm. Bull.*, *34*, 650-655.
35. Tanksley, D. and C. Rick (1980): Genetics of esterases in species of Lycoporicon. Theor. Apple. Genetic.(56) 209-219.
36. Tanksley, S. and T. Orton, (1983): Isozymes in plant genetic and breeding. Part (B). Elsevier Science Bublishers B.V. Amesterdam.
37. Venkatesan, N., D. Punithavathi, V. Arumugam, (2000): Curcumin prevents adriamycin nephrotoxicity in rats. *Br. J. Pharmacol.* 129: 231–234.
38. Visser G. J., L. Peters, J. J. Rasker (1992): Rheumatologists and their patients who seek alternative care: an agreement to disagree. *Br J Rheumatol*.; 31: 485 – 489.
39. Wang C. C., L. G. Chen, L.T. Lee, and L. L.Yang (2003): Effect of 6-gingerol, an antioxidant from ginger, on inducing apoptosis in human leukemic HL-60 cells. *In Vivo*; 17: 641–5.
40. Wheeler D.L., R. A. Vander Griend, T. J. Wronski, G. J. Miller, E. E. Keith and J. E. Graves (1995): The short- and long-termeffects ofmethotrexate on the rat skeleton. *Bone.* 16:215 – 21.
41. Winocur G, Vardy J, Binns MA, Kerr L, Tannock I. The effects of the anti-cancer drugs, methotrexate and 5-fluorouracil, on cognitive function in mice. Pharmacol Biochem Behav 2006;1:66–75.
42. Xian C.J.; J.C. Cool, M.A. Scherer, C.E. Macsai, C. Fan, M. Covino, *et al*. (2007): Cellular mechanisms for methotrexate chemotherapy-induced bone growth defects. *Bone*; 41: 842–50.
43. Yang G.Y., C. Gong, Qin Z.Yew., Y. Mao and A.L. Bertz (1998): Inhibition of TNFalpha attenuates infarct Volume and ICAM-1 expression in ischemic mouse brain. *Neuroreport* 9, 2131- 2134.

12/12/2012

Investigation of STATCOM Ability in Voltage Support

Mojtaba Shirvani [1], Ahmad Memaripour [2], Mostafa Abdollahi [3], Asadollah Salimi [4]

[1,2,3,4] Department of Electrical Engineering, Boroujen Branch, Islamic Azad University, Boroujen, Iran
mo_shirvani@yahoo.com

Abstract: This paper presents the application of static synchronous compensator (STATCOM) to control of voltage at a multi-machine power system installed with STATCOM as cast study. STATCOM is installed in one bus to control of voltage in the proposed bus. An optimization technique is used to tune the proposed STATCOM controllers. Several time-domain simulation tests visibly show the validity of proposed methods in voltage control and also damping of power system oscillations.

[Mojtaba Shirvani, Ahmad Memaripour, Mostafa Abdollahi, Asadollah Salimi. Investigation of STATCOM Ability in Voltage Support. *Life Sci J* 2012;9(4):4887-4891] (ISSN:1097-8135). http://www.lifesciencesite.com.734

Keywords: Static Synchronous Compensator, Voltage Support, Multi-machine Power System, Particle Swarm Optimization.

1. Introduction

The rapid development of the high-power electronics industry has made Flexible AC Transmission System (FACTS) devices viable and attractive for utility applications. FACTS devices have been shown to be effective in controlling parameters of power system and also in damping power system oscillations. In recent years, new types of FACTS devices have been investigated that may be used to increase power system operation flexibility and controllability, to enhance system stability and to achieve better utilization of existing power systems.

It has long been recognized that the steady-state transmittable power can be increased and the voltage profile along the line also can be controlled by appropriate reactive shunt compensation. The purpose of this reactive compensation is to change the natural electrical characteristics of the transmission line to make it more compatible with the prevailing load demand. Thus, shunt connected, fixed or mechanically switched reactors are applied to minimize line overvoltage under light load conditions, and shunt connected, fixed or mechanically switched capacitors are applied to maintain voltage levels under heavy load condition [1].

The ultimate objective of applying reactive shunt compensation such as STATCOM in a transmission system is to increase the transmittable power. This may be required to improve the steady-state transmission characteristics as well as the stability of the system. Var compensation is thus used for voltage regulation at the midpoint (or some intermediate) to segment the transmission line and at the end of the (radial) line to prevent voltage instability, as well as for dynamic voltage control to increase transient stability and damp power oscillations.

The static synchronous compensator (STATCOM) is one of the most important FACTS devices and it is based on the principle that a voltage-source inverter generates a controllable AC voltage source behind a transformer-leakage reactance so that the voltage difference across the reactance produces active and reactive power exchange between the STATCOM and the transmission network. The STATCOM can be used for dynamic compensation of power systems to provide voltage support [2, 3]. Also it can be used for transient stability improvement by damping low frequency power system oscillations [4-7].

The objective of this paper is to investigate the ability of STATCOM for voltage support. Particle Swarm Optimization (PSO) method as a meta-heuristic optimization method is considered for tuning the parameters of STATCOM. A multi-machine power system installed with STATCOM is considered as case study. Simulation results show the validity of STATCOM in voltage support at bulk electric power systems.

2. Test system

A multi machine power system installed with STATCOM is considered as case study. The proposed test system is depicted in Figure 1. The system data can be found in [8]. In this paper, turbine-governor system is also modeled to eliminate steady state error of responses.

2.1. Dynamic model of the system with STATCOM

The nonlinear dynamic model of the system installed with STATCOM is given as (1). The

dynamic model of the system installed with STATCOM is completely presented in [1].

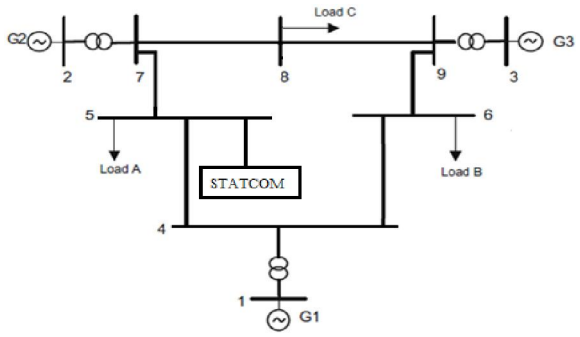


Figure 1. Multi-machine electric power system installed with STATCOM

$$\begin{cases} \dot{\omega} = (P_m - P_e - D\omega)/M \\ \dot{\delta} = \omega_0(\omega - 1) \\ \dot{E}'_q = (-E_q + E_{fd})/T'_{do} \\ \dot{E}_{fd} = (-E_{fd} + K_a(V_{ref} - V_t))/T_a \\ \dot{V}_{dc} = (K_r(V_{ref} - V) - b_{SVC})/T_r \end{cases} \quad (1)$$

Where, δ: Rotor angle; ω: Rotor speed (pu); P_m: Mechanical input power; P_e: Electrical output power (pu); M: System inertia (Mj/MVA); E'_q: Internal voltage behind x'_d (pu); E_{fd}: Equivalent excitation voltage (pu); T'_{do}: Time constant of excitation circuit (s); K_a: Regulator gain; T_a: Regulator time constant (s); V_{ref}: Reference voltage (pu); V_t: Terminal voltage (pu).

By controlling m_E, the output voltage of the shunt converter is controlled. By controlling δ_E, exchanging active power between the STATCOM and the power system is controlled.

2.2. STATCOM controllers

In this paper two control strategies are considered for STATCOM:

i. DC-voltage regulator

ii. Bus-voltage regulator

STATCOM has two internal controllers which are bus voltage controller and DC voltage regulator. A DC capacitor is installed behind the STATCOM; this capacitor is used to provide the reference voltage for PWM performance. In order to maintaining the voltage of this capacitor, a DC-voltage regulator is incorporated. DC-voltage is regulated by modulating the phase angle of the shunt converter voltage. This controller is commonly a PI type controller. A bus voltage controller is also incorporated based on STATCOM. The bus voltage controller regulates the voltage of bus where the STATCOM is installed.

The most important subject is to tuning the STATCOM controller parameters. The system stability and suitable performance is guaranteed by appropriate adjustment of these parameters. Many different methods have been reported for tuning STATCOM parameters so far. In this paper, an optimization method named is considered for tuning STATCOM parameters. In the next section an introduction about the proposed optimization method is presented.

3. Particle Swarm Optimization

PSO was formulated by Edward and Kennedy in 1995. The thought process behind the algorithm was inspired by the social behavior of animals, such as bird flocking or fish schooling. PSO is similar to the continuous GA in that it begins with a random population matrix. Unlike the GA, PSO has no evolution operators such as crossover and mutation. The rows in the matrix are called particles (same as the GA chromosome). They contain the variable values and are not binary encoded. Each particle moves about the cost surface with a velocity. The particles update their velocities and positions based on the local and global best solutions as below.

$$V_{m,n}^{new} = w \times V_{m,n}^{old} + \Gamma_1 \times r_1 \times (P_{m,n}^{local\ best} - P_{m,n}^{old}) + \Gamma_2 \times r_2 \times (P_{m,n}^{global\ best} - P_{m,n}^{old}) \quad (2)$$

$$P_{m,n}^{new} = P_{m,n}^{old} + \Gamma\, V_{m,n}^{new} \quad (3)$$

Where:

$V_{m,n}$ = particle velocity

$P_{m,n}$ = particle variables

W= inertia weight

r_1, r_2 = independent uniform random numbers

$\Gamma_1 = \Gamma_2$ = learning factors

$P_{m,n}^{local\ best}$ = best local solution

$P_{m,n}^{global\ best}$ = best global solution

The PSO algorithm updates the velocity vector for each particle then adds that velocity to the particle position or values. Velocity updates are influenced by both the best global solution associated with the lowest cost ever found by a particle and the best local solution associated with the lowest cost in the present population. If the best local solution has a cost less than the cost of the current global solution, then the best local solution replaces the best global solution. The particle velocity is reminiscent of local minimizes that use derivative information, because velocity is the derivative of position. The advantages of PSO are that it is easy to implement and there are few parameters to adjust. The PSO is able to tackle tough cost functions with many local minima [9].

4. STATCOM tuning based on PSO

In this section the parameters of the STATCOM controllers are tuned by using PSO. The optimum values of controllers which minimize different performance indices are accurately computed using PSO. The performance index is considered as (4). In fact, the performance index is the Integral of the Time multiplied Absolute value of the Error (ITAE).

$$ITAE=\int_0^t t\sum_{i=1}^{3}|\Delta\omega_i|dt+\int_0^t t\sum_{i=1}^{9}|\Delta v_i|dt \qquad (4)$$

Where, $\Delta\omega$ shows the frequency deviations and Δv shows the voltage of buses. To compute the optimum parameter values, different faults are assumed in all buses and then the minimum solution is chosen as final solution. The results are listed in Table 1.

Table 1. Optimal parameters of STATCOM

	gain	value
PI controller of voltage	Proportional gain	1.42
	Integrator gain	0.57
PI controller of DC link	Proportional gain	25.1
	Integrator gain	0.38

5. Simulation results

The proposed STATCOM is evaluated on the test system given in section 2. The disturbance is provided by a 10 cycles three phase short circuit in bus 1. This disturbance shows a large signal disturbance in power systems. The simulation results are presented in Figures 2-6. Where, solid line indicates the system installed with STATCOM and dashed line shows the system without STATCOM.

The STATCOM is installed in bus 5 and it is expected that voltage of bus 5 be controlled. In this regard, the voltage of all buses are depicted in Figures 2-10. It is clearly seen that the STATCOM can successfully control the voltage of bus 5. It is also seen that STATCOM has a positive effect on the voltage of rest buses. Where, the voltage profile in all buses is better than the system without STATCOM. The STATCOM is installed to control of voltage, bus is has an effect on the system dynamic performance. It is seen that the system with STATCOM is more stable than system without STATCOM. STATCOM affects the system damping and the oscillations are rapidly damped out with being of STATCOM.

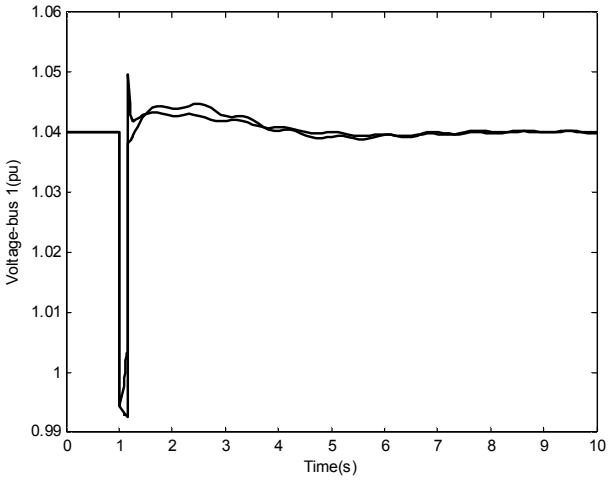


Figure 2. Voltage bus 1 following disturbance

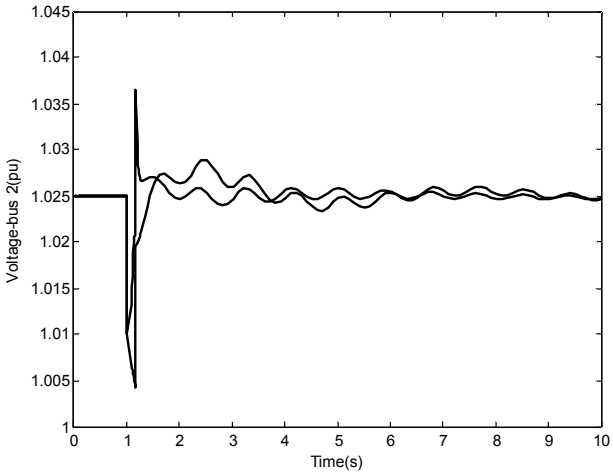


Figure 3. Voltage bus 2 following disturbance

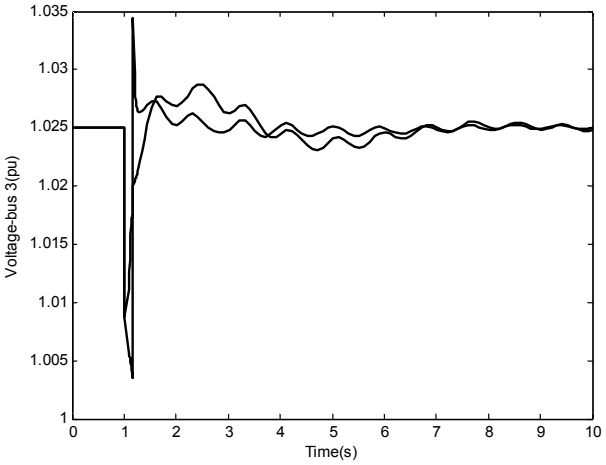


Figure 4. Voltage bus 3 following disturbance

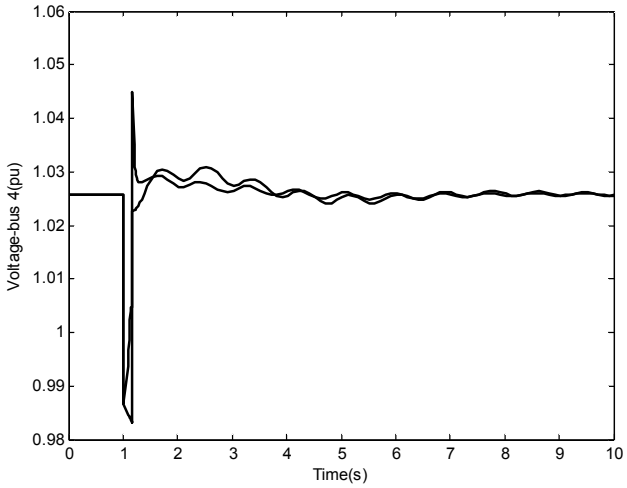


Figure 5. Voltage bus 4 following disturbance

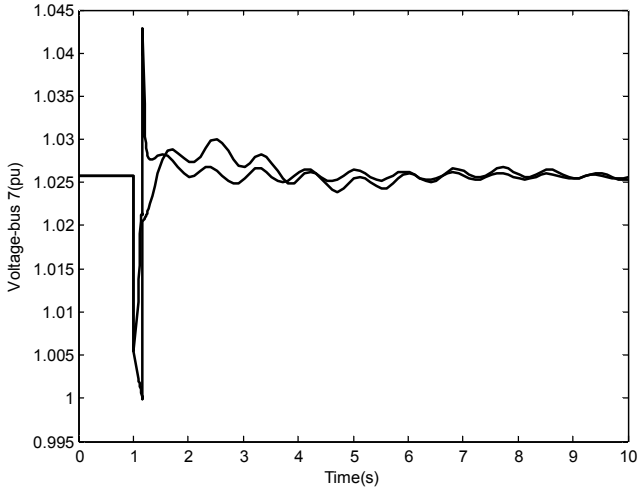


Figure 8. Voltage bus 7 following disturbance

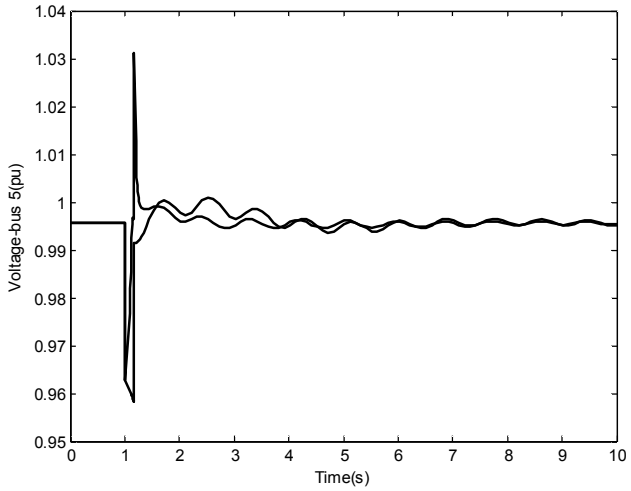


Figure 6. Voltage bus 5 following disturbance

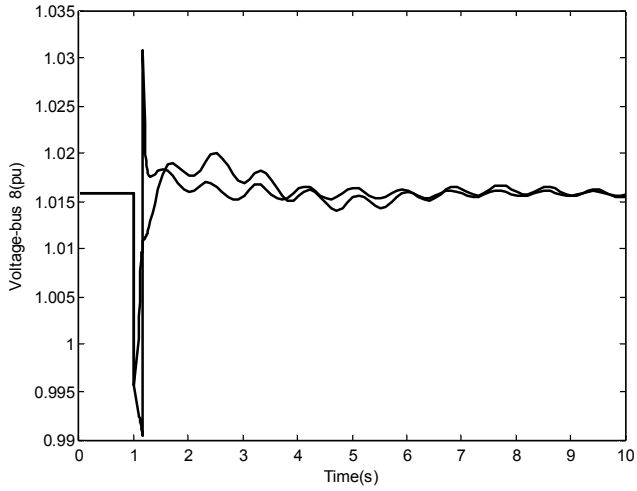


Figure 9. Voltage bus 8 following disturbance

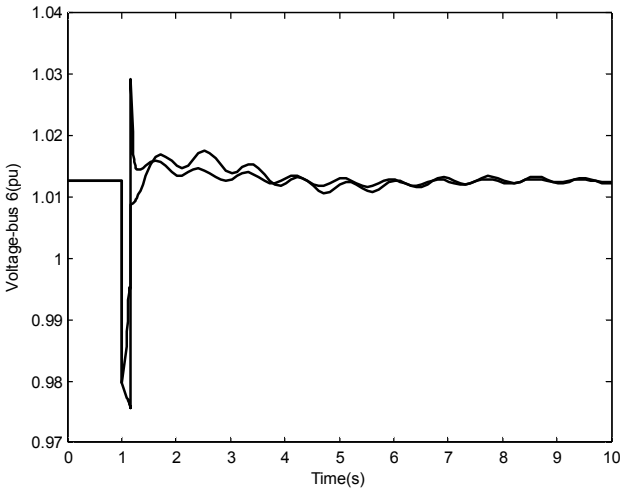


Figure 7. Voltage bus 6 following disturbance

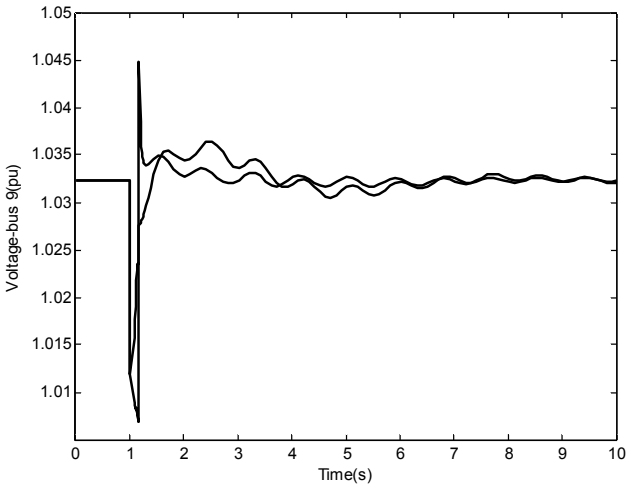


Figure 10. Voltage bus 9 following disturbance

6. Conclusions

The application of STATCOM in voltage support was investigated in this paper. A multi-machine electric power system installed with STATCOM was assumed to demonstrate the ability of STATCOM in voltage support. The parameters of the proposed STATCOM were tuned by using a Meta-heuristic optimization method. The proposed optimization procedure guaranteed the solution to reach a suitable and optimal response. Three phase short circuit was considered as disturbance, this is the worst case fault in power system which was assumed to evaluate the dynamic performance of system. Simulation results demonstrated that the designed STATCOM can guarantee the robust stability and robust performance under large signal disturbances.

Corresponding Author:
Mojtaba Shirvani
Department of Electrical Engineering,
Boroujen Branch, Islamic Azad University,
Boroujen, Iran.
E-mail: mo_shirvani@yahoo.com

References

[1] N.G. Hingorani, L. Gyugyi, M. El-Hawary. Understanding FACTS: concepts and technology of flexible AC transmission systems: IEEE press New York; 2000.
[2] A.H.M.A. Rahim, M.F. Kandlawala. Robust STATCOM voltage controller design using loop-shaping technique. Electric Power Systems Research. 68 (2004) 61-74.
[3] C.A. Cañizares, C. Cavallo, M. Pozzi, S. Corsi. Comparing secondary voltage regulation and shunt compensation for improving voltage stability and transfer capability in the Italian power system. Electric Power Systems Research. 73 (2005) 67-76.
[4] M.A. Abido. Analysis and assessment of STATCOM-based damping stabilizers for power system stability enhancement. Electric Power Systems Research. 73 (2005) 177-85.
[5] S.A. Al-Baiyat. Power system transient stability enhancement by STATCOM with nonlinear H∞ stabilizer. Electric Power Systems Research. 73 (2005) 45-52.
[6] W. Du, X. Wu, H.F. Wang, R. Dunn. Feasibility study to damp power system multi-mode oscillations by using a single FACTS device. International Journal of Electrical Power & Energy Systems. 32 (2010) 645-55.
[7] M.A. Furini, A.L.S. Pereira, P.B. Araujo. Pole placement by coordinated tuning of Power System Stabilizers and FACTS-POD stabilizers. International Journal of Electrical Power & Energy Systems. 33 (2011) 615-22.
[8] P.W. Sauer, M. Pai. Power system dynamics and stability: Prentice Hall New Jersey; 1998.
[9] R.L. Haupt, S.E. Haupt. Practical genetic algorithms: Wiley-Interscience; 2004.

12/12/2012

Angiogenesis and current approaches to deal with its misregulation in related diseases

Mohammadhossein Hassanshahi [1], Samira Khabbazi [2], Ali Reza Hassanshahi[3], omid mohammadi [4]

Hassanshahi.hossein@gmail.com

Abstract: Angiogenesis, which is the formation of blood vessels from pre-existing vessels, normally supply nutrition and oxygen to cells and tissues. In medicine point of view, regulation of angiogenesis is disrupted in many diseases such as cancers, psoriasis, age related macular degeneration, diabetes, proliferative retinopathies and rheumatoid arthritis. How to suppress, control and regulate the angiogenesis have been very challenging tasks in order to provide better and more effective treatments for related patients. With this regard, anti-angiogenic therapy has been considered as a potential approach to do so. However, anti-angiogenic agents are not completely safe and present side effects. Therefore, many attentions have been paid to understand more about molecular and cellular mechanisms involved in angiogenesis in order to prevent many life-threating side effects of anti-angiogenic agents. It may lead to discovering more desirable drugs to tackle angiogenesis. This review aims to give an overview about what angiogenesis is as well as present the most important factors involved in angiogenesis. It also attempts to describe current approaches and challenges in controlling angiogenesis.
[Mohammadhossein Hassanshahi, Samira Khabbazi, Ali Reza Hassanshahi, omid mohammadi. **Angiogenesis and current approaches to deal with its misregulation in related diseases**. *Life Sci J* 2012;9(4):4892-4902] (ISSN:1097-8135). http://www.lifesciencesite.com. 735

Keywords: Angiogenesis, pre-existing, anti-angiogenic, blood, misregulation

Introduction

Angiogenesis is defined as forming new blood vessels from pre-existing vascular system (Logan-Collins, Lowy et al. 2008). Angiogenesis normally occurs in adult (e.g. wound healing) and during embryogenesis. However, angiogenesis is not always regulated properly, so that in many diseases such as cancers, psoriasis, age related macular degeneration, diabetes, proliferative retinopathies and rheumatoid arthritis misregulation of angiogenesis is seen (table 1) (Folkman 2007). Clinically speaking, improving and expanding knowledge regarding angiogenesis and in order to understand more about cellular and molecular mechanism which are involved in regulation and control the angiogenesis could lead scientists to provide more effective treatment for affected patients. This paper aims to address the most important factors involved in angiogenesis and present some current approaches impacting pathological angiogenesis in order to control the angiogenesis.

To date, many molecules have been recognized which are associated with the process of angiogenesis such as: vascular endothelial growth factors (VEGFs) and VEGF receptors (VEGFRs), immunoglobulin families (e.g. JAM-C) (Lamagna, Hodivala-Dilke et al. 2005), remodeling and guidance molecules including slit/robo, delta/notch, ephrin/eph (Huang, Xu et al. 2010), netrin/UNC-DCC (Freitas, Larrivee et al. 2008) and semaphorin/ plexin (Kim, Oh et al. 2011), extracellular matrix (ECM) proteins, such as collagens and fibronectin, adhesion molecules of the cadherin (VE and N-cadherin) (Gerhardt, Liebner et al. 1999) and integrin (e.g. αvβ3, α5β1) (Reardon, Neyns et al. 2011), homeobox gene products (e.g. HoxD3 and HoxB3) (Kodama, Sakai et al. 2009), transcription factors (e.g. HIF1a, NFjB) and inhibitors (e.g. Id1, 2), plasminogen activators/ inhibitors (uPA, PAI1) and 3 receptors(uPAR), matrix-degrading proteinases, in particular matrix metalloproteinases (e.g. MMP2, 9), MMP inhibitors (i.e. TIMPs) (Ruegg and Mutter 2007).

Angiogenesis has been considered as a multidisciplinary field of study for scientists. In other words, this subject encompasses various areas such as cell and molecular biology, physiology, experimental pathology, mouse genetics and drug development in order to understand the pathological and physiological aspect of angiogenesis (Ruegg and Mutter 2007). Imbalanced controlling angiogenesis can lead to aberrant blood vessel development (Gagne, Akalu et al. 2004). Even though angiogenesis therapy has been a target to treat cancer or many diseases related to angiogenesis process, in clinical trials the anticipated effects of anti- angiogenic therapy has been moderated (Wang, Cao et al. 2010).

1. Vascular endothelial growth factor (VEGF)

As mentioned earlier, there are various types of angiogenic factors which are correlated somehow in angiogenesis. However, vascular endothelial growth factor (VEGF) has been regarded as the most important factor in pathological and physiological status (Dong, Han et al. 2007). It has been stated that in approximately 30-60% of solid tumours VEGFs are expressed, while in other diseases (e.g. renal cell carcinoma) this number goes up to 100% (Longo and

Gasparini 2007). Detection of VEGFs routinely can be performed through various approaches such as ELISA and Immunohistochemistry (table 2).

The VEGF family is correlated with great number of diseases and consist of 6 members namely VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor (PlGF). They are varied doe to either processing or alternative splicing. They bind to two types of receptors: VEGFRs and co-receptors (e.g. neuropilins) (Tugues, Koch et al. 2011). Among these six growth factors, VEGF-A is the most well characterized member so that due to alternate gene splicing, six isoforms of VEGF-A have been recognized (Sun and Schiller 2007) .VEGF promotes endothelial cell survival in newly formed vessels, stimulates endothelial cell proliferation (Wang, Li et al. 2008) and induces proteases which are engaged in the degradation of the extracellular matrix (ECM) required for endothelial cell migration (Sun and Schiller 2007). In addition, it functions as stimulator of vascular permeability (Zhang, Parangi et al. 2009).

There are three receptors in the family, VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1) and VEGFR-3 (Flt-4). VEGF-A, VEGF-B and PIGF are able to bind VEGFR-1(Sun and Schiller 2007) . Inactivation of VEGFR-1 or VEGFR-2 is likely to be cause of embryonic lethality demonstrating the vital role of those receptors in the development of the vascular system. It is believed that VEGFR-2 is the main mediator of the permeability enhancing effects of VEGF, angiogenic and mitogenic (Ferrara, Hillan et al. 2004).

VEGFR-1 plays an important role in angiogenesis development (Ferrara 2001). It has been suggested that VEGFR-1 acts as a regulator in angiogenesis process. Nevertheless, the exact function of the receptor has not been completely defined. The larger part of angiogenic effects of VEGF-A including, migration and survival, endothelial cell proliferation, invasion and enhanced vascular permeability is mediated by VEGFR-2 (Sun and Schiller 2007). It is believed that VEGFR-2 is the main regulator of angiogenesis (Dvorak 2002). It has been proved that tumour cells, circulating endothelial cells (CECs), intratumoural endothelial cells and endothelial progenitor cell (CEPs) express VEGFR-1 and VEGFR-2 (Longo and Gasparini 2007).

In adults, expression of VEGFR-3 is restricted to the lymphatic endothelium, while during development it is expressed in all endothelial cells (Tammela, Zarkada et al. 2008). It is also indicated that the expression of such receptors may be restricted to some fenestrated vascular endothelium in adolescents (Mouawad, Spano et al. 2009). Up regulation of VEGFR-3 can be seen in wounds and tumours in angiogenic blood vessels which might contribute in solid tumour growth and tumour angiogenesis (Petrova, Bono et al. 2008). However, in terms of angiogenesis, Zhang and colleagues show that VEGFR-3 ligand-binding is not necessary for angiogenesis but is an essential element for lymphangiogenesis (Zhang, Zhou et al. 2010)

2. Neuropilins and heparan sulphate as two co-receptors

Neuropilins and heparan sulphate (HS) are known as two types of VEGF co-receptores (Sorensen, Emblem et al. 2012). Neuropilin 1 (NP1) acts as a receptor for the heparin-binding isoforms of VEGF so that VEGF-A165 is presented to VEGFR2 by NP1, resulting in VEGFR-2 signalling (Ferrara, Hillan et al. 2004). While, neuropilin 2 (NRP2), which is one homologous of two homologous of NRP, acts as a receptor for class3 semaphorins which are involved with development of nervous and vascular system (Sorensen, Emblem et al. 2012) . NPR 1 binds PIGF, VEGF-B, while NRP2 binds VEGF-C and VEGF-A 145 (Klagsbrun, Takashima et al. 2002). Due to anti-angiogenic activity of semaphorins, It has been shown that semaphorins (Class III) may be considered as a target for anti-angiogenc therapies (Neufeld, Sabag et al. 2012).

Heparan sulphate receptors are complex polysaccharides which are involved in variety of biological functions such asmetastasis, angiogenesis and tumourogeneis (Wegrowski and Maquart 2004). Due to binding of extracellular matrix proteins, growth factors (e.g. VEGFs) and enzymes to heparin sulphates and consequently triggering chemical response (Dredge, Hammond et al. 2010), heparan sulfate receptors are potentially considered as co-receptors for VEGFs in angiogenesis. In mice with expression of VEGF-A isoform, association of heparan sulphate in regulating retinal vascular development has been investigated (Tugues, Koch et al. 2011).

3. Integrins

Integrins are heterodimeric transmembrane receptors and consist of α andβ chains. Although various integrins such as α1β1, α2β1, α3β1, α5β1, αvβ3, αvβ5 are associated in angiogenesis, the role of αvβ5, as many studies have shown, is crucially important in new vessel formation (Gagne, Akalu et al. 2004). Integrins have been studied as factors which affect anticancer therapy. Integrins antagonists can be combined with cytotoxic anticancer therapy by which cancerous and endothelial cells can be destroyed (Huveneers, Truong et al. 2007). The role of integrin family in angiogenesis is critical. Integrin family regulates various important function related to solid tumours such as initiation, progression and metastasis (Desgrosellier and Cheresh 2010). They are considered as a crucial target in cancer therapy (Jin and Varner

2004). Various functions in tumor cell are regulated owing to integrin signaling. These functions include proliferation, migration, survival and invasion of tumour. Moreover, it has been determined that there is an association between expression of specific integrin and increase in disease development, resulting in reduction in patient survival (Desgrosellier and Cheresh 2010). Proliferation and survival of integrin are prevented by integrin antagonist activities through disruption of binding extracellular matrix (ECM) to their receptors. As a result, tumourgenesis can be suppressed by such integrin antagonists (Huveneers, Truong et al. 2007). Therefore, it may be worth to investigate how integrin antagonists affect tumour, as well as its microenvironment

Debates about the role of integrins in angiogenesis are still controversial. Some believe that integrins activity can decrease angiogenesis if such receptors are blocked (Weis and Cheresh 2011), while others like Reynolds and colleagues support that integrins may play an opposite role in angiogenesis . They indicate that pathological angiogenesis is enhanced in mice lacking β3 and β5 integrins (Reynolds, Wyder et al. 2002). As many studies have proved, inhibition or blocking of integrins are affective strategies to cease the angiogenesis, Avaraamides and colleagues state that the ablation of αv integrins in mice has no effect in angiogenesis and present two possibilities accordingly. One is αv integrins functions as negative regulators of angiogenesis and second is angiogenesis occur in animal with lack of αv integrins as the result of compensatory changes in VEGF signaling (Avraamides, Garmy-Susini et al. 2008). Taken together, it seems that more investigations are required to understand the precise mechanism of such receptors.

4. Anti-angiogenic therapies and current challenges

There are three different ways that anti- angiogenic therapy may influence on tumour vessels: A) as the result of pruning of abnormal vessels, the structure of some remained vessels may be formed as normal vessel so that it leads to proper blood perfusion B) increasing necrosis, hypoxia and invasion due to excessive blood vessels abnormality and decrease in perfusion C) no effect. (Sorensen, Emblem et al. 2012). Additionally, three types of angiogenesis inhibitors mostly interact with growth factors (table 3) (Folkman 2007).Different anti-angiogenic agents are being used in clinical base. Table 4 and 5 show anti-angiogenic drugs in clinical development and clinical trials.

bFGF, basic fibroblast growth factor; EGF, epidermal growth factor; HIF1α, hypoxia-inducible factor 1α; ID1, inhibitor of DNA binding 1, dominant negative helix–loop–helix protein; PDGF, platelet-derived growth factor; TIMP2, tissue inhibitor of metalloproteinase 2; TGFα, transforming growth factor-α; VEGF, vascular endothelial growth factor.

ALS, amyotrophic lateral sclerosis (or Lou Gehrig's disease); ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; CLL, chroniclymphocytic leukaemia; CML, chronic myeloid leukaemia; CNS, central nervous system; GIST, gastrointestinal stromal tumour; MDS, myelodysplastic syndromes; NSCLC,non-small-cell lung cancer; NHL, non-Hodgkin's lymphoma; SCLC, small-cell lung cancer; VHL, von Hippel Lindau.

Gange and colleagues state that anti-angiogenic therapy provides many advantages such as elevated specificity for cancerous tissues. However, anti- angiogenic therapy is not "problem free", so that using such drugs can result to disruption of normal processes such as wound healing, pregnancy, the menstrual cycle, mucosal regeneration, bone remodelling may occur as consequences of anti-angiogenic therapy (Gagne, Akalu et al. 2004).
Treating patients with anti- angiogenic drugs may lead to some side effects such as hypertension, proteinuria, thrombosis,etc (Mitchell and Bryan 2010) (Table 6).

ECM, extracellular matrix; TF, tissue factor; TM, thrombomodulin; NO, nitric oxide; PGI2 , prostacyclin; VEGF, vascular endothelial growth factor.

Bergers and Hanahan propose that there are two modes of resistance to angiogenic therapy namely: pre-existing or intrinsic indifference and evasive resistance which is an adaption to circumvent the specific angiogenic blockade. Evasive (adaptive) resistance consists of accentuated invasiveness of tumour cells into local tissue to co-opt normal vasculature, revascularization consequent to upregulation of alternative pro-angiogenic signals, increased metastatic seeding and tumour cell growth in lymph nodes and distant organs, and protection of the tumour vasculature either by recruiting pro-angiogenic inflammatory cells or by increasing protective pericyte coverage (Bergers and Hanahan 2008). By contrast, in intrinsic resistance, even though molecular and cellular mechanism intrinsic resistance might be similar to previous resistance, it has been suggested that certain tumours seem to have a pre-existing tumour microenvironment which can transfer indifference due to treatment history, their stage of progression, host genotype or genomic constitution (Bergers and Hanahan 2008). A great number of patients (e.g., pancreatic cancer patients) are intrinsically resistant to anti- angiogenic therapy. A great deal of information from preclinical investigations has shown that the mechanism of resistance to anti- angiogenic drug in

evasive and intrinsic refractoriness is referred to the tumour cells themselves (Loges, Schmidt et al. 2010).

4.1. Anti-angiogenic drugs and chemotherapy

In order to enhance the effect of treatment in patients there is another strategy which is combination of anti VEGF drugs and chemotherapeutic agents. In this approach, anti-VEGF materials are responsible to normalize tumour vessels, resulting in improvement in blood flowing to tumours. Therefore, after normalisation of blood vessels, access of chemotherapeutic drugs to cancerous cell can be facilitated (De Bock, Cauwenberghs et al. 2011). It should also be noted that, however, anti- angiogenic drugs might close the blood barrier in tumour vessels, resulting in limitation in access of chemotherapeutic drugs to the tumours (van Kempen and Leenders 2006).In principal, the effects of chemotherapy should be decreased when an anti VEGF is administrated owing to reduction in drug supply by elimination of blood vessels (Sorensen, Emblem et al. 2012). For combination of anti- angiogenic drugs with chemotherapeutic drugs or radiation therapy, it is crucial to consider the mechanism of action of radiation or specific chemotherapeutic compounds (Gagne, Akalu et al. 2004). There is another point related to combination of anti-angiogenic therapy with chemotherapy which is about optimal scheduling such combination and its requirement to understand how long they remain in that state and the knowledge of the time window (the vessels are normalized) (van Kempen and Leenders 2006)

4.2. Molecular targets in angiogenesis

Tyrosine kinase inhibitors

Tyrosine kinases have been used as efficient therapeutic target to deal with angiogenesis process. Of many angiogenic receptors tyrosine kinase inhibitors (TKIs) can mention to Sarofenib and Sunitinib, which target VEGF path way and have been efficient to teat different types of cancers (Verheul and Pinedo 2007). One type of TKIs, for instance, is known as TKI-31. TKI-31 has been known as a tyrosine kinase inhibitor which inhibits c-Kit and c-Src on molecular base, platelet-derived growth factor receptor beta (PDGFRb), vascular endothelial growth factor receptor 2 (VEGFR2) (Zhong, Guo et al. 2006). A main problem correlated with progression of TKI is the "plethora of existing kinases". As a consequence, develop of drug for particular target is quite challenging which may lead to emerge of side effects (Ruegg and Mutter 2007). Another point related to TKIs which should be considered is about the relapse of disease in patients who undergo treatment with TKIs (Ellis, Hammers et al. 2009).

Peptide

High specificity and low toxicity of peptides have considered them as an crucial therapeutics which are being tested in diseases related to angiogenesis (Rosca, Koskimaki et al. 2011). Peptides also possess other advantages in this matter, including their solubility, lack of immune response in the host cell, stability and enhanced bio-avalibility. Of such peptides can mention to leucine rich repeat 5 of decorin, N terminal of parathyroid hormone, arginine rich N terminus of endostatin, pigment epithelium derived factor and peptides derived from type 1 repeat of thrombospondin, alpha 4 and beta 1 chains of laminin (Sulochana and Ge 2007). A research study, for instance, determines that P14 (residues 43-57 of PEDF molecule) and P18 (residues 39-57 of PEDF molecule) block endothelial cell chemotaxis. It also demonstrates that P 18 and P23 (residues 34-57 of PEDF molecule) stimulate apoptosis. This study proposes that P 18 can be regarded as novel agent for anti-angiogenic therapy for patients with renal and prostate cancer (Mirochnik, Aurora et al. 2009). Most of angiogenesis inhibitors are "endogenous short anti-angiogeneic peptides" such as tumstatin peptide, endostatin fragments and PF-4 peptide (Yi, Cho et al. 2009).

Monoclonal antibodies

One of the main advantages of monoclonal antibodies (mAbs) is their high specificity and binding their target with high affinity (Ruegg and Mutter 2007). Due to being produced of mAbs in mice, it is required that mAbs be humanised in order to decrease the risk of response from human immune system. In patients, antibodies are administrated every 2-3 weeks owing to their long half-lives (Ruegg and Mutter 2007). There are various research studies which have addressed the effect of monoclonal antibodies on angiogenesis. As an example, Nielsen and colleagues used a monoclonal antibody, Trastuzumab (Herceptin), which is used against extra cellular domain of the HER2 protein. This protein is involved in metastasis of breast cancer in women (Nielsen, Andersson et al. 2009). The exact mechanism of Trastuzumab as an anti-tumour drug has not been cleared yet. Nevertheless, in angiogenesis point of view, it has been stated that Trastuzumab reduce angiogenesis activity through activationg of angiogenic agents and reduction of VEGFs (Kaneda, Okamoto et al. 2012) . Monoclonal antibodies like other strategies have disadvantages. For example, a research study indicates that monoclonal antibody against PIGF has side effects in mouse due to minimal expression of PIGF of normal cells (unlike VEGF) (Kerbel 2008). Many of monoclonal antibodies have been approved by the FDA in order to be used in clinical trials (table 7) (Liu, Pop et al. 2008). Also, table (8) presents monoclonal

antibodies which are being tested in USA to be used for treatment of related diseases (Liu, Pop et al. 2008).

mAb, monoclonal antibody; IgG, immunoglobulin G; RCC, renal cell cancer; EGFR, epidermal growth factor receptor; NSCLC, non-small cell lung cancer; CTLA-4, cytotoxic T lymphocyte antigen-4; CTCL, cutaneous T-cell lymphoma; CLL, chronic lymphocytic leukemia; NHL, non-Hodgkin's lymphoma; Ep-CAM, epithelial cell adhesion molecule; MUC1, mucin receptor; CEA, carcinoembryonic antigen; IL-6, interleukin-6; CNS, central nervous system; PSMA, prostate-specific membrane antigen; Her-1, epidermal growth factor receptor; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; DR5, death receptor; TRAIL-R1, tumor necrosis factor apoptosis-inducing ligand receptor 1; HLA DRB, human leukocyte antigen (Liu, Pop et al. 2008). Another example of using monoclonal antibody with significant effects is using those antibodies in patients suffering non-Hodgkin's lymphoma (NHL) (Fanale and Younes 2007). For this type of patients Rituximab (anti-CD20 antibody) has respond well. Whereas, rituximab has been used as front line treatment of funicular lymphoma, and in patients with intermediate and aggressive B-cell lymphomas, rituximab can be combined with chemotherapy (Fanale and Younes 2007).

Of main issues related to optimising the efficacy of monoclonal antibody therapy can mention to duration of use, continuing to refine its optimal timing with chemotherapy, whether it should be continued at disease progression and maintenance therapy (Fanale and Younes 2007).

Table 1 Angiogenesis-dependent diseases (Taken from Folkman 2007).

Disease	Symptoms
Diabetic retinopathy	Loss of vision
Rheumatoid arthritis	Pain and immobility from destroyed cartilage
Atherosclerotic plaques	Chest pain, dyspnoea
Endometriosis	Abdominal pain from intraperitoneal bleeding
Crohn's disease	Intestinal bleeding
Psoriasis	Persistent severe itching
Uterine fibroids	Vaginal bleeding, abdominal pain
Benign prostatic hypertrophy	Urinary retention
Cancer	Bleeding, thrombosis, anaemia, abdominal ascites, bone pain, seizures from cerebral oedema around a tumour and others
Benign prostatic hypertrophy	Urinary retention

Table 2 Common methods which are used to detect vascular endothelial growth factor (VEGF) detection methods (Adapted from Longo and Gasparini 2007

Method and description	Comments
Immunohistochemistry (IHC) detects VEGF protein expression in whole tissue sections (usually formalin-Wxed, paraYn-embedded tissue)	Possible to diVerentiate between tumor and non-tumor VEGF expression Simple to perform Most common detection method No standardized methodology or scoring procedure Results variable and subjective
Enzyme-linked immunosorbent assay (ELISA) and chemiluminescence immunosorbent assay (ICMA) detect VEGF protein expression in tissue homogenate (fresh-frozen tissue), serum, or plasma	Serum and plasma measurements convenient vs. tissue samples Can be automated for high throughput Cannot distinguish between tumor and non-tumor sources of VEGF Circulating VEGF may be bound to serum proteins and unavailable to ELISA antibodies Serum measurements may be confounded by release of VEGF from platelets
Western blotting detects VEGF protein expression in tissue homogenate (fresh-frozen tissue)	Cannot distinguish between tumor and non-tumor sources of VEGF Less simple to perform than IHC
In situ hybridization (ISH) detects VEGF mRNA in whole tissue sections (ideally, fresh-frozen tissue)	Can distinguish between tumor and non-tumor VEGF expression May not relate directly to VEGF protein expression Less simple to perform than IHC
Northern blotting detects VEGF mRNA from tissue homogenates (fresh-frozen tissue)	Cannot distinguish between tumor and non-tumor VEGF expression May not relate directly to VEGF protein expression Less simple to perform than IHC
Reverse-transcription polymerase chain reaction (RT-PCR) detects VEGF mRNA in tissue homogenates (usually fresh-frozen)	Quantitative method that can be automated for high-throughput Cannot distinguish between tumor and non-tumor sources of VEGF Sensitive to contamination May not relate directly to VEGF protein expression
RNase protection assay detects VEGF mRNA in cellular extracts (tissue or circulating)	Cannot distinguish between tumor and non-tumor VEGF expression May not relate directly to VEGF protein expression Relatively complex to perform

Table 3 | Three types of angiogenesis inhibitors (Taken from Folkman 2007).

Mechanism	Drug	Action
Type 1 Blocks one main angiogenic protein	Avastin (Avastin; Genentech) B	Blocks VEGF
	VEGF Trap (Regeneron Pharmaceuticals)	Blocks VEGF
Type II Blocks two or three main angiogenic proteins	Sutent (Sutent; Pfizer)	Downregulates VEGF receptor 2, PDGF receptor, cKIT receptor
	Tarceva (Tarceva; Genentech, OSI Pharmaceuticals, Roche)	Downregulates VEGF production, bFGF production, TGF by tumour cell
Type III Blocks a broad range of angiogenic regulators	Endostatin	Downregulates VEGF, bFGF, bFGF receptor, HIF1α, EGF receptor, ID1, neuropilin Upregulates thrombospondin 1, maspin, HIF1α, TIMP2
	Caplostatin	Broad anti-angiogenic and anticancer spectrum

Table 4 Current anti- angiogenic agents in clinical development (Taken from Sun and Schiller 2007).

Drug	Target	Phase of development	Toxicities
Monoclonal antibodies Bevacizumab (Avastin; Genentech)	VEGF-A	Approved for advanced NSCLC and metastatic CRC; phase II SCLC in development	Hypertension, proteinuria, arterial and venous thromboembolic events,and hemorrhage
Tyrosine kinase inhibitors • SU11248 (Sutent,sunitinib; Pfizer)	VEGFR-1–3, PDGFR, c-kit, FLT-3	Approved for mRCC and metastatic GIST; phase II NSCLC ongoing	Fatigue, asthenia, rash, yellow skin discoloration, de pigmentation, neutropenia, hypertension, stomatitis
• PTK787 (vatalanib; Novartis)	VEGFR-1–3, PDGFR-β, c-kit, cFms	Phase II NSCLC	Fatigue, nausea/vomiting, dizziness, hypertension, ataxia, dyspnea
• ZD6474 (Zactima; Astra-Zeneca)	VEGFR-2, EGFR	Phase II/III NSCLC	Diarrhea, rash, hypertension, proteinuria, QTc prolongation, transaminitis
• AZD2171 (Astra-Zeneca)	VEGF-1–3	Phase II/III NSCLC	Fatigue, nausea/vomiting, diarrhea, abdominal pain, hemorrhage, hypoglycemia, hypertension
• BAY 93-4006 (sorafenib; Bayer)	Raf-1, VEGFR-2, -3, PDGFR, c-kit	Approved for mRCC; phase II NSCLC ongoing	Fatigue, diarrhea, transaminitis, skin toxicity, hypertension
• GW-786034 (Glaxo-Smith-Kline)	VEGFR-1–3	Phase I	Fatigue, nausea/vomiting, anorexia, hypertension, hair depigmentation
• CP-547,632(Pfizer)	VEGFR-2	Phase I	Fatigue, nausea/vomiting, diarrhea, rash, dry mouth
• AG-013736 (Pfizer)	VEGFR-1–3, PDGFR, c-kit	Phase II NSCLC	Fatigue, nausea, hypertension, transaminitis, seizure, pancreatitis, stomatitis, hemoptysis

NSCLC: non-small cell lung cancer; CRC: colorectal cancer; mRCC: metastatic renal cell carcinoma; GIST: gastrointestinal stromal tumor.

Table 5 | Anti-angiogenic drugs which are approved for clinical use and phase of clinical trials for other indications (Adapted from Folkman 2007).

Drug (Trade name;company)	Approved	Phase III	Phase II	Phase I
Bortezomib (Velcade; Millennium Pharmaceuticals)	Multiple myeloma (2003)	NSCLC, multiple myeloma, NHL	Multiple myeloma, NHL, NSCLC, lymphoma, gliomas, melanoma, Waldenstrom's macroglobinaemia, prostate, head and neck, breast, liver, nasopharyngeal, gastric, pancreatic, colorectal, cervical/vaginal cancer, and others	Lymphoma, myelodysplasia, multiple myeloma, NHL, solid tumours, head and neck, cervical, colorectal, ovarian, prostate cancer, and others
Thalidomide (Thalomid; Celgene Corporation)	Multiple myeloma (2003)	Multiple myeloma, brain metastases, SCLC, NSCLC, prostate, kidney, ovarian, hepatocellular	Soft tissue sarcoma, multiple myeloma, ALS,melanoma, neuroendocrine tumours, leukaemia,glioma, glioblastomas, paediatric	Solid tumours, glioma

		cancer	neuroblastoma, NSCLC, NHL, paediatric solid tumours, myelofibrosis, myelodysplastic syndrome, AML, CLL, SCLC, Hodgkin's disease, paediatric brain stem, liver, colorectal, kidney, neuroendocrine, endometrial,thyroid, uterine, ovarian cancer, and others	
Bevacizumab (Avastin; Genentech)	Colorectal cancer (2004), lung cancer (2006)	NSCLC, GIST, diabetic retinopathy, vascular occlusions, retinopathy of prematurity, colorectal, breast, ovarian, peritoneal, pancreatic, prostate, kidney cancer	Glioblastoma, glioma, mesothelioma, NSCLC, AML, CLL, CML, lymphoma, angiosarcoma, melanoma, billary tumours, SCLC, Kaposi's sarcoma, sarcomas, NHL, carcinoid, oesophagogastric, gastric, renal cell, head and neck, rectal, hepatocellular, bladder, pancreatic, gall bladder, breast, neuroendocrine, cervical, ovarian, endometrial cancer, and others	NSCLC, pancreatic, solid tumours, head and neck tumours, VHL, retinal tumours
Erlotinib (Tarceva; Genentech, OSI Pharmaceuticals, Roche)	Lung cancer (2004)	NSCLC, colorectal, pancreatic, ovarian, head and neck, oral cancer	NSCLC, mesothelioma, glioblastoma, glioma, gall bladder, GIST, biliary tumours, bladder cancer prevention, malignant peripheral nerve sheath tumours, endometrial, colorectal, pancreatic, breast, renal cell, prostate, ovarian, head and neck, gastric/oesophageal, liver cancer, and others	NSCLC, glioblastoma, solid tumours, colorectal, pancreatic, head and neck cancer
Pegaptanib (Macugen; OSI Pharmaceuticals)	Age-related macular degeneration (2004)			
Endostatin (Endostar)	Lung cancer (2005)			
Sorafenib (Nexavar;	Kidney cancer	Kidney, melanoma,	Melanoma, glioblastoma, GIST, SCLC, thyroid, neuroendocrine, mesothelioma, soft tissue	Solid tumours, melanoma, glioblastoma, NHL, glioma
Onyx Pharmaceuticals)	(2005)	hepatocellular cancer	sarcoma, NSCLC, CLL, multiple myeloma, cholangiocarcinoma, NHL, kidney, colorectal, prostate, ovarian, peritoneal, pancreatic, breast, gastric, head and neck, uterine, gall bladder, bladder cancer, and others	multiple myeloma, Kaposi's sarcoma, ALL, CML, MDS
Lenalidomide (Revlimid;Celgene Corporation)	Myelodysplastic syndrome (2005)	Multiple myeloma, myelodysplastic syndrome	NSCLC, NHL, multiple myeloma, CLL, myelofibrosis, myelodysplastic syndrome, glioblastoma, ocular melanoma, AML, mantle-cell lymphoma, Waldenstrom's macroglobinaemia, ovarian/ peritoneal, thyroid, prostate cancer	Multiple myeloma, prostate cancer, melanoma, myelodysplastic syndrome, solid tumours, paediatric CNS tumours
Sunitinib (Sutent; Pfizer)	GIST, kidney cancer (2006)	Renal cell cancer, GIST	Melanoma, VHL/solid tumour, NSCLC, GIST, hepatocellular, colorectal, prostate, breast, renal cell, gastric, neuroendocrine cancer, and others	Melanoma, solid tumours,colorectal, breast cancer
Ranibizumab (Lucentis; Genentech)	Age-related macular degeneration (2006)			

Table 6. Molecular mechanisms of toxicity of angiogenesis inhibition (Taken from Verheul and Pinedo 2007).

Toxicity	Possible underlying mechanism
Bleeding, disturbed wound healing	Platelet dysfunction; decreased expression of endothelial TF
Thrombotic events	Endothelial cell apoptosis; lack of endothelial cell renewal leading to exposure of the ECM to the circulating blood (results in platelet activation); increased TF expression; reduced TM and NO; direct platelet activation
Hypertension	Decreased NO and/or PGI2 production; inappropriate density of vessels (arterioles and capillaries); vascular stiffness; disturbed endothelin function
Hypothyroidism	Disturbed thyroid cell function; reduced vascularity of thyroid
Fatigue	Hypothyroidism
Proteinuria and oedema	Podocyte dysfunction owing to VEGF blockade; hypertension
Leukopenia, lymphopenia and immunomodulation	Inhibition of haematopoiesis and/or myelopoiesis; impaired dendritic cell function
Dizziness, nausea, vomiting and diarrhoea	Mucosa disturbance
Skin toxicity including rash and hand-foot syndrome	Epidermal cell apoptosis

Table 7. Monoclonal antibodies approved by the FDA for cancer therapy (Taken from Liu, Pop et al. 2008).

Generic (Trade mark)	Target antigen	Isotype	Species	Payload	Mechanism of action	Antitumor therapeutic activity	FDA year of approving
Rituximab (Rituxan™)	CD20	IgG1 k	Chimeric	–	Induction of apoptosis, ADCC, CDC,chemosensitization	Low grade B-cell NHL	1997
Trastuzumab (Herceptin™)	Her-2/neu	IgG1 k	Humanized	–	ADCC, chemosenzitation, CCA, inhibition of angiogenesis	Her-2 overexpressed metastatic breast cancer	1998
Alemtuzumab (Campath-1H™)	CD52	IgG1 k	Humanized	–	ADCC, CDC	B-cell CLL	2001
Cetuximab (Erbitux™)	EGFR (Her-1)	IgG1 k	Chimeric	–	Inhibition of angiogenesis, chemosensitization and radiosensitization, CCA, ADCC	Metastatic colorectal cancer, head and neck cancers	2004
Bevacizumab (Avastin™)	VEGF	IgG1 k	Humanized	–	Inhibition of angiogenesis	Colorectal cancer	2004
Panitumumab (Vectibix™)	EGFR	IgG2 k	Human	–	Inhibition of cell growth, induction of apoptosis, decreased proinflamatory cytokines and VEGF production	Metastatic colorectal cancer	2006
Gemtuzumab ozogamicin (Mylotarg™)	CD33	IgG4 k	Humanized	Calicheamicin	Double-stranded DNA breaks and cellular death induced by payload after intracellular hydrolysis	CD33+ relapsed AML	2000
Ibritumomab tiuxetan (Zevalin™)	CD20	IgG1 k	Murine	90-yttrium	Cellular death induced by β-emitter, induction of apoptosis, ADCC, CDC	Low grade or follicular, relapsed or refractory, CD20+ B-cell NHLs; Rituximab-refractory follicular NHL	2002
Tositumomab (Bexxar™)	CD20	IgG2a λ	Murine	131-iodine	Cellular death induced by γ-emitter, induction of apoptosis, ADCC, CDC	Relapsed CD20+ B-cell NHL; Rituximab-refractory NHL	2003

Table 8. Monoclonal antibodies currently undergoing human clinical testing for cancer therapy in the USA (Adapted from Liu, Pop et al. 2008)

Name	Target antigen	Isotype	Specie	Payload	Antitumor therapeutic activity	Clinical trial phase
WX-G250 (Rencarex™)	Carbonic anhidrase IX	IgG1	Chimeric	–	RCC stages I–III	III
Ipilimumab (MDX-010)	CTLA-4	IgG1 k	Human	–	Malignant melanoma stages I–III Metastatic pancreatic cancer	III II
Zanolimumab (HuMax-CD4)	CD4	IgG1 k	Human	–	Malignant melanoma stages I–III, CTCL	III
Ofatunumab (HuMax-CD20)	CD20	IgG1	Human	–	Drug resistant B-cell CLL, low grade or follicular, relapsed or refractory, CD20+ B-cell NHLs; Rituximab-refractory follicular NHL	III
ch14.18	Ganglioside GD2	IgG	Chimeric	–	Neuroblastoma	III
Zalutumumab (HuMax-EGFr)	EGFR	IgG1 k	Human	–	Head and neck cancer (squamous cell type), NSCLC stage III	III
Oregovomab (B43.13, OvaRex™)	CA 125	IgG1	Murine	–	Advanced varian cancer (epithelial adenocarcinoma type)	III
Edrecolomab (IGN-101, Panorex™)	17-1A (Ep-CAM)	IgG2a	Murine	Colorectal cancer		III
131I-chTNT-1/B (Cotara™)	DNA/histone H1 complex	IgG	Human	131-iodine	Solid tumors	III
Pemtumomab (R-1549,Theragyn™	MUC1	IgG1	Murine	90-yttrium	Ovarian cancer	III
Lintuzumab (SGN-33)	CD33	IgG1	Humanized	–	AML	III
Labetuzumab (hMN14, CEAcide™)	CEA	IgG	Murine	–	Colorectal cancer	III
Catumaxomab (Removab™)	Ep-CAM and CD3	Trifunctional bispecifc	Rat × mouse quadroma	–	Gastric adenocarcinoma, advanced ovarian cancer, malignant ascites	II
CNTO 328 (cCLB8)	IL-6	IgG1 k	Chimeric	–	Hormone refractory prostate cancer, relapsed or refractory multiple myeloma, advanced RCC	II
3F8	Ganglioside GD2	IgG3	Murine	131-iodine	CNS or leptomeningeal neoplasms	II/III
177Lu -J591	PSMA	IgG	Murine	177-	Metastatic androgen-	II
Nimotuzumab	EGFR (Her-1)	IgG1 k	Humanized	–	Metastatic colorectal cancer, lung cancer, metastatic pancreas cancer	II
SGN-30	CD30	IgG1	Chimeric	–	Relapsed or refractory Hodgkin's lymphoma, anaplastic large cell lymphoma	II
Ticilimumab (CP-675206)	CTLA-4	IgG2	Human	–	Metastatic melanoma	II
Daclizumab (Zenapax™)	CD25	IgG1	Humanized	–	Adult T-cell leukemia/lymphoma	II
Epratuzumab (hLL2, LymphoCide™)	CD22	IgG1	Humanized	–	Relapsed CD22+ ALL, NHL	II
90Y-Epratuzumab	CD22	IgG1	Humanized	90-yttrium	NHL	II
Galiximab (IDEC-114)	CD80	IgG1 λ	Chimeric	–	Relapsed or refractory NHLs	II
MDX-060	CD30	IgG1 k	Human	–	Relapsed or refractory Hodgkin's lymphoma	II
CT-011	B7	IgG	Humanized	–	Diffuse large B-cell lymphoma	II
CS-1008	DR5	IgG	Humanized	–	Metastatic pancreatic cancer	II
SGN-40	CD40	IgG	Humanized	–	Diffuse large B-cell lymphoma	II
Mapatumumab (TRM-1)	TRAIL-R1	IgG1	Human	–	Relapsed or refractory multiple myeloma, NHL, colorectal cancer, NSCLC.	II
Apolizumab (Hu1D10, Remitogen™)	HLA DRB	IgG1	Humanized	–	B-cell NHL, refractory CLL	II
Volociximab (M200)	α5β1 integrin	IgG4	Chimeric	–	Advanced ovarian cancer	II

References:

1. Avraamides, C. J., B. Garmy-Susini, et al. (2008). "Integrins in angiogenesis and lymphangiogenesis." Nat Rev Cancer **8**(8): 604-617.
2. Bergers, G. and D. Hanahan (2008). "Modes of resistance to anti-angiogenic therapy." Nat Rev Cancer **8**(8): 592-603.
3. De Bock, K., S. Cauwenberghs, et al. (2011). "Vessel abnormalization: another hallmark of cancer? Molecular mechanisms and therapeutic implications." Current Opinion in Genetics & Development **21**(1): 73-79.
4. Desgrosellier, J. S. and D. A. Cheresh (2010). "Integrins in cancer: biological implications and therapeutic opportunities." Nat Rev Cancer **10**(1): 9-22.
5. Dong, X., Z. C. Han, et al. (2007). "Angiogenesis and antiangiogenic therapy in hematologic malignancies." Crit Rev Oncol Hematol **62**(2): 105-118.
6. Dredge, K., E. Hammond, et al. (2010). "The PG500 series: novel heparan sulfate mimetics as potent angiogenesis and heparanase inhibitors for cancer therapy." Investigational New Drugs **28**(3): 276-283.
7. Dvorak, H. F. (2002). "Vascular permeability factor/vascular endothelial growth factor: a critical cytokine in tumor angiogenesis and a potential target for diagnosis and therapy." J Clin Oncol **20**(21): 4368-4380.
8. Ellis, L., H. Hammers, et al. (2009). "Targeting tumor angiogenesis with histone deacetylase inhibitors." Cancer Lett **280**(2): 145-153.
9. Fanale, M. A. and A. Younes (2007). "Monoclonal antibodies in the treatment of non-Hodgkin's lymphoma." Drugs **67**(3): 333-350.
10. Ferrara, N., K. J. Hillan, et al. (2004). "Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer." Nat Rev Drug Discov **3**(5): 391-400.
11. Folkman, J. (2007). "Angiogenesis: an organizing principle for drug discovery?" Nat Rev Drug Discov **6**(4): 273-286.
12. Freitas, C., B. Larrivee, et al. (2008). "Netrins and UNC5 receptors in angiogenesis." Angiogenesis **11**(1): 23-29.
13. Gagne, P., A. Akalu, et al. (2004). "Challenges facing antiangiogenic therapy for cancer: impact of the tumor extracellular environment." Expert Rev Anticancer Ther **4**(1): 129-140.
14. Gerhardt, H., S. Liebner, et al. (1999). "N-cadherin expression in endothelial cells during early angiogenesis in the eye and brain of the chicken: relation to blood-retina and blood-brain barrier development." Eur J Neurosci **11**(4): 1191-1201.
15. Huang, L., Y. Xu, et al. (2010). "Effect of Robo1 on retinal pigment epithelial cells and experimental proliferative vitreoretinopathy." Invest Ophthalmol Vis Sci **51**(6): 3193-3204.
16. Huveneers, S., H. Truong, et al. (2007). "Integrins: signaling, disease, and therapy." Int J Radiat Biol **83**(11-12): 743-751.
17. Jin, H. and J. Varner (2004). "Integrins: roles in cancer development and as treatment targets." British Journal of Cancer **90**(3): 561-565.
18. Kaneda, H., I. Okamoto, et al. (2012). "Reversible posterior leukoencephalopathy syndrome and trastuzumab." Invest New Drugs **30**(4): 1766-1767.
19. Kerbel, R. S. (2008). "Molecular origins of cancer: Tumor angiogenesis." New England Journal of Medicine **358**(19): 2039-2049.
20. Kim, J., W. J. Oh, et al. (2011). "Semaphorin 3E-Plexin-D1 signaling regulates VEGF function in developmental angiogenesis via a feedback mechanism." Genes Dev **25**(13): 1399-1411.
21. Klagsbrun, M., S. Takashima, et al. (2002). "The role of neuropilin in vascular and tumor biology." Adv Exp Med Biol **515**: 33-48.
22. Kodama, A., H. Sakai, et al. (2009). "Immunohistochemical demonstration of angiogenesis-associated homeobox proteins in canine vascular tumours." J Comp Pathol **141**(2-3): 199-203.
23. Lamagna, C., K. M. Hodivala-Dilke, et al. (2005). "Antibody against junctional adhesion molecule-C inhibits angiogenesis and tumor growth." Cancer Res **65**(13): 5703-5710.
24. Liu, X. Y., L. M. Pop, et al. (2008). "Engineering therapeutic monoclonal antibodies." Immunol Rev **222**: 9-27.
25. Logan-Collins, J. M., A. M. Lowy, et al. (2008). "VEGF expression predicts survival in patients with peritoneal surface metastases from mucinous adenocarcinoma of the appendix and colon." Ann Surg Oncol **15**(3): 738-744.
26. Loges, S., T. Schmidt, et al. (2010). "Mechanisms of resistance to anti-angiogenic therapy and development of third-generation anti-angiogenic drug candidates." Genes Cancer **1**(1): 12-25.
27. Longo, R. and G. Gasparini (2007). "Challenges for patient selection with VEGF inhibitors." Cancer Chemother Pharmacol **60**(2): 151-170.
28. Mirochnik, Y., A. Aurora, et al. (2009). "Short pigment epithelial-derived factor-derived peptide inhibits angiogenesis and tumor growth." Clin Cancer Res **15**(5): 1655-1663.
29. Mitchell, D. C. and B. A. Bryan (2010). "Anti-angiogenic therapy: adapting strategies to overcome resistant tumors." J Cell Biochem **111**(3): 543-553.
30. Mouawad, R., J. P. Spano, et al. (2009). "Tumoural expression and circulating level of

VEGFR-3 (Flt-4) in metastatic melanoma patients: correlation with clinical parameters and outcome." Eur J Cancer **45**(8): 1407-1414.
31. Nielsen, D. L., M. Andersson, et al. (2009). "HER2-targeted therapy in breast cancer. Monoclonal antibodies and tyrosine kinase inhibitors." Cancer Treatment Reviews **35**(2): 121-136.
32. Neufeld, G., A. D. Sabag, et al. (2012). "Semaphorins in angiogenesis and tumor progression." Cold Spring Harb Perspect Med **2**(1): a006718.
33. Petrova, T. V., P. Bono, et al. (2008). "VEGFR-3 expression is restricted to blood and lymphatic vessels in solid tumors." Cancer Cell **13**(6): 554-556.
34. Reardon, D. A., B. Neyns, et al. (2011). "Cilengitide: an RGD pentapeptide alphanubeta3 and alphanubeta5 integrin inhibitor in development for glioblastoma and other malignancies." Future Oncol **7**(3): 339-354.
35. Reynolds, L. E., L. Wyder, et al. (2002). "Enhanced pathological angiogenesis in mice lacking beta3 integrin or beta3 and beta5 integrins." Nat Med **8**(1): 27-34.
36. Rosca, E. V., J. E. Koskimaki, et al. (2011). "Anti-angiogenic peptides for cancer therapeutics." Curr Pharm Biotechnol **12**(8): 1101-1116.
37. Ruegg, C. and N. Mutter (2007). "Anti-angiogenic therapies in cancer: achievements and open questions." Bull Cancer **94**(9): 753-762.
38. Sorensen, A. G., K. E. Emblem, et al. (2012). "Increased survival of glioblastoma patients who respond to antiangiogenic therapy with elevated blood perfusion." Cancer Res **72**(2): 402-407.
39. Sulochana, K. N. and R. Ge (2007). "Developing antiangiogenic peptide drugs for angiogenesis-related diseases." Curr Pharm Des **13**(20): 2074-2086.
40. Sun, S. and J. H. Schiller (2007). "Angiogenesis inhibitors in the treatment of lung cancer." Crit Rev Oncol Hematol **62**(2): 93-104.
41. Tammela, T., G. Zarkada, et al. (2008). "Blocking VEGFR-3 suppresses angiogenic sprouting and vascular network formation." Nature **454**(7204): 656-660.
42. Tugues, S., S. Koch, et al. (2011). "Vascular endothelial growth factors and receptors: anti-angiogenic therapy in the treatment of cancer." Mol Aspects Med **32**(2): 88-111.
43. van Kempen, L. C. L. and W. P. J. Leenders (2006). "Tumours can adapt to anti-angiogenic therapy depending on the stromal context: Lessons from endothelial cell biology." European Journal of Cell Biology **85**(2): 61-68.
44. Verheul, H. M. and H. M. Pinedo (2007). "Possible molecular mechanisms involved in the toxicity of angiogenesis inhibition." Nat Rev Cancer **7**(6): 475-485.
45. Wang, C., J. Cao, et al. (2010). "Recombinant vascular basement membrane derived multifunctional peptide blocks endothelial cell angiogenesis and neovascularization." J Cell Biochem **111**(2): 453-460.
46. Wang, S., X. Li, et al. (2008). "Control of endothelial cell proliferation and migration by VEGF signaling to histone deacetylase 7." Proc Natl Acad Sci U S A **105**(22): 7738-7743.
47. Wegrowski, Y. and F. X. Maquart (2004). "Involvement of stromal proteoglycans in tumour progression." Crit Rev Oncol Hematol **49**(3): 259-268.
48. Weis, S. M. and D. A. Cheresh (2011). "alphav Integrins in Angiogenesis and Cancer." Cold Spring Harb Perspect Med **1**(1): a006478.
49. Yi, Z. F., S. G. Cho, et al. (2009). "A novel peptide from human apolipoprotein(a) inhibits angiogenesis and tumor growth by targeting c-Src phosphorylation in VEGF-induced human umbilical endothelial cells." Int J Cancer **124**(4): 843-852.
50. Zhang, L., F. Zhou, et al. (2010). "VEGFR-3 ligand-binding and kinase activity are required for lymphangiogenesis but not for angiogenesis." Cell Res **20**(12): 1319-1331.
51. Zhang, X., S. Parangi, et al. (2009). "Regulation of VEGF-Induced Vascular Permeability and VEGFR2 Signaling by Thrombospondin-1." Laboratory Investigation **89**: 344a-344a.
52. Zhong, L., X. N. Guo, et al. (2006). "TKI-31 inhibits angiogenesis by combined suppression signaling pathway of VEGFR2 and PDGFR beta." Cancer Biology & Therapy **5**(3): 323-330.

12/12/2012

Calculation of generation system reliability index: Loss of Load Probability

Hasan Fayazi Boroujeni [1], Meysam Eghtedari [2], Mostafa Abdollahi [3], Elahe Behzadipour [4]

[1,2,3,4] Department of Electrical Engineering, Boroujen Branch, Islamic Azad University, Boroujen, Iran
hasanfayaziboroujeni@gmail.com

Abstract: Generation system reliability is an important factor in the long term planning for future system capacity expansion to make sure that the total installed capacity is sufficient to support demand. The planning process utilizes reliability indices as criteria to decide on new investments in new generation capacities. Generation system reliability is evaluated by using different indexes. In this paper, loss load of probability (LOLP) is simulated to evaluate the system reliability. Effects of the system parameters such as forced outage rate (FOR) are tested on the LOLP index.

[Hasan Fayazi Boroujeni, Meysam Eghtedari, Mostafa Abdollahi, Elahe Behzadipour. **Calculation of generation system reliability index: Loss of Load Probability.** *Life Sci J* 2012;9(4):4903-4908] (ISSN:1097-8135). http://www.lifesciencesite.com. 736

Keywords: Generation System Reliability, Loss of Load Probability, Capacity Outage Probability Table, Analytically Method.

1. Introduction

Electricity has been the driving force for economies of the world and provides day-to-day necessity for the population in the world. The generation, transmission and retailing of electricity have existed hundreds of years in providing the much needed electricity. Due to the nature of electricity systems, the variable demand at every moment needs to be met by consistent electricity supply in making sure the continuous availability of the resources. Not meeting the demand in any case will lead to a huge loss of income to the generators as well as to the consumers. The reliability of the generation, transmission and distribution of electricity in this sense is crucial for the continuous supply of electricity to meet the demand.

A modern power system is complex, highly integrated and very large. Fortunately, the system can be divided into appropriate subsystems or functional areas that can be analyzed separately [1]. These functional areas are generation, transmission and distribution. The function of the generation system is to make sure enough capacity is available to meet the load/demand at any time. Transmission and distribution systems need to be reliable in making sure the electricity generator can be delivered to the consumers. System planners have been assigned the role of planning for forecasting the load into the future and plant capacity addition to meet the load and provide a level of reliability in case some of the plants are out on maintenance or breakdown. Probabilistic method is often used to determine the system reliability and the system reliability can be summed up into a single value, the reliability indices. Reliability studies are conducted for two purposes. Long-term evaluations are performed to assist in system planning and short-term evaluations to assist in day to day operating decisions. In short, these reliability indices (for long-term evaluations) are used by system planners and the authorities to decide on and advice for new investments in building new generation capacities [1].

Generation system reliability is an important aspect in the planning for future system capacity expansion. It provides a measurement of reliability or adequacy to make sure that the total generation system capacity is sufficient to provide adequate electricity when needed [1].

In this paper an important reliability index LOLP is evaluated for generation system. The proposed index is simulated by using analytically method. Effects of changing system parameters such as FOR are tested on the LOLP.

2. Generation system reliability

Reliability has been and always is one of the major factors in the planning, design, operation, and maintenance of electric power system. Generation system reliability focuses on the reliability of generators in the whole electric power system where electric power is produced from the conversion process of primary energy (fuel) to electricity before transmission. The generation system is an important part of the electricity supply chain and it is crucial that enough electricity is generated at every moment to meet the demand. Generating units will occasionally fail to operate and the system operator has to make sure that enough reserve is available to be operated when this situation happens [2-31].

Reliability of the generation system is divided into adequacy and security [32]. System adequacy relates to the existence of sufficient generators within

the system to satisfy the consumer load demand or system operational constraints. System adequacy is associated with static conditions of the system and do not include system disturbances. System security on the other hand relates to the ability of the system to respond to disturbances arising within the system. Therefore system security is associated with the response of the system to whatever perturbation it is subjected to. In this study, the reliability evaluations will be focused on the generation system adequacy and will not take into account system security.

The basic modeling approach for the generating system adequacy assessment consists of three parts as shown in Figure 1. The generation and load models are convolved to form an appropriate risk model where the element of interest is the risk of generation capacity less than the load. In short, adequacy evaluation of generation systems consists of four general steps as Figure 1.

(i) Create a generation capacity model; (ii) create a load model; (iii) combined the generation capacity model with load model to obtain a risk model and (iv) calculating indexes.

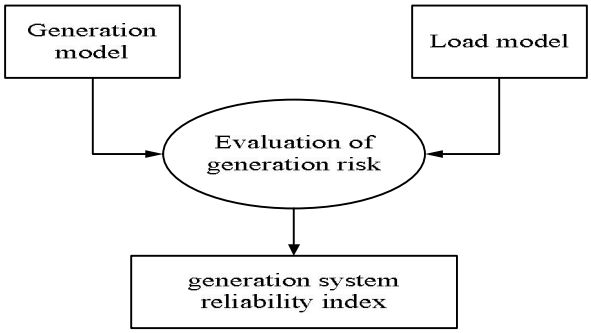


Figure 1. Generation reliability evaluation process

Analytical methods or Monte Carlo simulation [33] can be used to calculate the reliability indices. Analytical techniques represent the system by analytical models and evaluate the indices from these models using mathematical solutions. Monte Carlo simulations, on the other hand estimate the indices by simulating the actual process and random behavior of the system, treating the problem as a series of experiments. The reliability indices obtained indicate the ability of the generating facilities to meet the system demand.

In the analytical method, the generating system model used for generation capacity adequacy assessment is a Capacity Outage Probability Table (COPT) which can be created using the recursive technique. As for the load model, the daily peak load or hourly load for a period of one year is normally used to form the Load Probability Table (LPT).

3. Load model

The load in a power system in any time period is a stochastic process, which is difficult to describe with a simple mathematical formula. Different models are created, starting from rimary load data and according to the need to calculate reliability. Primary load data will provide a minimum amount of data that is needed to establish an hourly chronological load profile. Most primary load data consist of the percentage of maximum monthly load or weekly load in a year, the load in 24 hours in a typical day in each season and the maximum load in each day in a week. With the percentages of these data available and the annual peak load known, the hourly chronological load profile can be established.

4. Forced Outage Rate

There are many concepts in reliability evaluation such as: failure rate, repair time, unavailability, forced outage rate (FOR) and etc. Unit unavailability is also known conventionally as “forced outage rate” (FOR), although the value is not a rate. The FOR is defined as below.

$$\text{FOR} = \frac{\text{Forced outage hours}}{\text{In service hours} + \text{Forced outage hours}} \quad (1)$$

The FOR is calculated for a long period of time (e.g. 365 days), is the same index as the unavailability.

5. Generation system reliability indices

The quantification of reliability is an important aspect of generation system reliability assessment. The measurement used to quantify reliability of a generation system is given various reliability indices. These reliability indices are used to assess the reliability performance of a generation system against some predetermined minimum requirements or reliability standards, compare alternative designs, identify weak spots and determine ways for correction in the generation system and to be integrated with costs and performance considerations for decision making. These indices are better understood as estimates of system-wide generation adequacy and not as absolute measures of system reliability [18].

Basically, system reliability evaluations can be divided into deterministic and probabilistic. The most common deterministic indices are the Reserve Margin and the largest set in the system. An important shortcoming of these methods is that they do not account for the stochastic nature of system behavior.

Probabilistic methods can provide more meaningful information to be used in design and resource in planning and allocation. There are two

approaches that use probabilistic evaluation. The analytical methods and Monte Carlo simulation as can be seen from Figure 2. The analytical methods represent the system by mathematical models and use direct analytical solutions to evaluate reliability indices from the model. As for the Monte Carlo simulation, reliability indices are estimated by simulating the actual random behavior of the system. So of the commonly used probabilistic reliability indices are Loss of Load Probability (LOLP), Loss of Load Expectation (LOLE), Loss of Energy Probability (LOEP), Loss of Energy Expectation (LOEE), Expected Energy Not Served (EENS), and Loss of Load Frequency (LOLF) and Loss of Load Duration (LOLD). Most of these indices are basically expected values of a random variable. Expectation indices provide valid adequacy indicators which reflect various factors such as system component availability and capacity, load characteristics and uncertainty, system configurations and operational conditions, etc [1]. Typical reliability indices used in power system evaluations and their categorizing is shown in Figure 2.

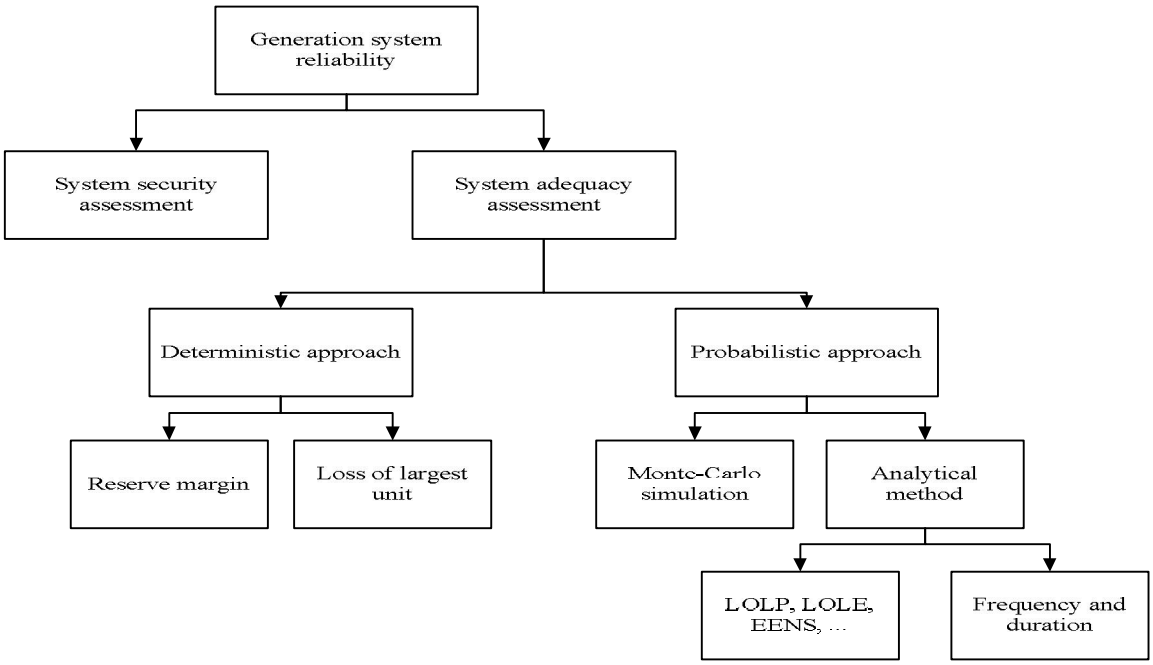


Figure 2. Generation system reliability assessment indices category

6. Loss of load probability

Loss of load occurs when the system load exceeds the generating capacity available for use. Loss of Load Probability (LOLP) is a projected value of how much time, in the long run, the load on a power system is expected to be greater than the capacity of the available generating resources. It is defined as the probability of the system load exceeding the available generating capacity under the assumption that the peak load of each day lasts all day [1].

LOLP is based on combining the probability of generation capacity states with the daily peak probability so as to assess the number of days during the year in which the generation system may be unable to meet the daily peak. LOLP can be calculated considering the daily peak loads for one year duration or sometimes on each hour's load for a 24 hours day. Therefore, the same system may have two or more values of LOLP depending on how the calculation is being done. The mathematical formula for calculation of LOLP is shown as below [1].

$$LOLP=\sum_j p[C_A=C_j].P[L>C_j]=\sum_j \frac{P_j.t_j}{100} \qquad (2)$$

where;

P: the probability; L: expected load; C_A: available generation capacity; C_j: remaining generation capacity; p_j: probability of capacity outage; t_j: percentage of time when the load exceeds C_j.

Alternatively, a load duration curve consists of daily peak loads arranged in descending order can be used to measure LOLP for long term generation capacity evaluation. The assumption used in this case is that the peak load of the day would last all day. The LOLP calculation is illustrated with a daily peak load curve in Figure 3 [1].

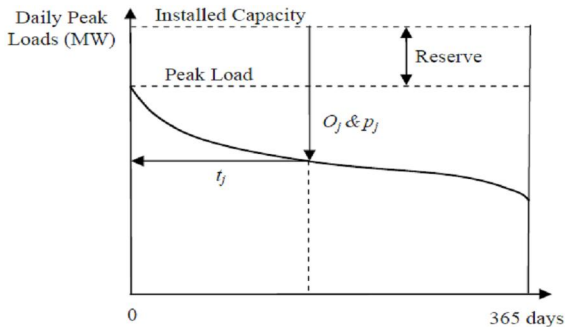


Figure 3. LOLP Calculation using Load Duration Curve

In the figure, the parameters are defined as following; O_j: is the magnitude of the j_{th} outage in the system; p_j: is the probability of a capacity outage of magnitude O_j; t_j: is the number of days that an outage of magnitude O_j would cause a loss of load in the system.

Capacity outage less than the amount of reserves will not contribute to a loss of load. When a particular capacity outage is greater than the reserve, the risk in this case will be $p_j \times t_j$.

7. Case study

In this section a numerical case study is carried out for reliability evaluation. Table 1 shows the proposed generation test system. This system contains four generation companies with six units. The system data and capacity of units are considered as typical. The load model is also considered as Figure 4.

Table 1. Generation system details

Generation Company	Number of units	Capacity of each unit (MW)	FOR
1	2	25	0.03
2	2	40	0.02
3	1	50	0.01
4	1	100	0.01

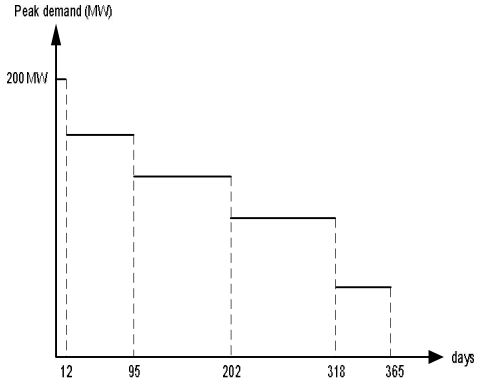


Figure 4. Daily peak demand of year

In this section LOLP index is calculated for the proposed test system. The procedure presented in section 6 is used to computing LOLP. In first the Capacity Outage Probability Table (COPT) is derived. Table 2 shows the COPT and the probability of different outages is listed.

Table 2. COPT for the test system

Capacity Outage (MW)	Probability
0	0.88565791683600000
25	0.05478296392800000
40	0.03614930272800000
50	0.00894603956400000
50	0.00084715923600000
65	0.00223603934400000
75	0.00055336327200000
80	0.00036887043600000
90	0.00036514447200000
90	3.45779280000000e-05
100	0.00894603956400000
100	8.55716400000000e-06
105	2.28167280000000e-05
115	2.25862560000000e-05
125	0.00055336327200000
130	3.72596400000000e-06
130	3.52836000000000e-07
140	0.00036514447200000
140	3.49272000000000e-07
150	9.03640360000000e-05
150	8.55716400000000e-06
155	2.30472000000000e-07
165	2.25862560000000e-05
175	5.58952800000000e-06
180	3.72596400000000e-06
180	3.56400000000000e-09
190	3.68832800000000e-06
190	3.49272000000000e-07
200	8.64360000000000e-08
205	2.30472000000000e-07
215	2.28144000000000e-07
230	3.76360000000000e-08
230	3.56400000000000e-09
240	3.52800000000000e-09
255	2.32800000000000e-09
280	3.60000000000000e-11
	Sum of probabilities=1

LOLP index is calculated as mentioned above. In this regard, the LOLP is obtained as below.

LOLP= 5.968138239671235e−004 (3)

In order to show the sensitivity of LOLP index to the system parameters, an evaluation is carried out and the results are listed in Table 3. It is seen that changing FORs and load has a direct effect of the reliability of generation system.

6. Conclusions

In this paper a commonly used reliability index of generation system LOLP was successfully calculated and evaluated. Different conditions and changing were considered. COPT was carried out and then the reliability calculated. Simulation results showed that changing components FOR and load level can directly affect of the system total reliability.

Table 3. Effect of changing parameters on the LOLP index

Parameter changing	LOLP
FOR unit 25 MW=0.01	5.043705938958903e-004
FOR unit 25 MW=0.05	6.889275761643836e-004
FOR unit 40 MW=0.05	7.691760586986303e-004
FOR unit 50 MW=0.05	7.519234803835614e-004
FOR unit 100 MW=0.1	0.0058208871141920000
Increasing load by 10% in all levels	8.500825094465753e-004
decreasing load by 10% in all levels	1.611630195397260e-004

Corresponding Author:
Hasan Fayazi Boroujeni
Department of Electrical Engineering,
Boroujen Branch, Islamic Azad University,
Boroujen, Iran.
E-mail: hasanfayaziboroujeni@gmail.com

References

[1] R. Billington, R.J. Ringlee, A.J. Wood, Power-system reliability calculations, 1973.
[2] R. Arya, S.C. Choube, L.D. Arya, Reliability evaluation and enhancement of distribution systems in the presence of distributed generation based on standby mode, International Journal of Electrical Power & Energy Systems, 43 (2012) 607-616.
[3] A.K. Azad, R.B. Misra, A new approach to evaluate reliability and production cost of a power generating system, Computers & Electrical Engineering, 22 (1996) 343-351.
[4] B. Banerjee, S.M. Islam, Reliability based optimum location of distributed generation, International Journal of Electrical Power & Energy Systems, 33 (2011) 1470-1478.
[5] J. Batut, J. Boisseau, E. Bourgade, M. Monti, Different approaches to reliability as applied to the generation — transmission system and its components, International Journal of Electrical Power & Energy Systems, 10 (1988) 168-173.
[6] R. Billinton, T.K.P. Medicherla, Effect of system peak load forecast uncertainty on annualized reliability indices of composite generation and transmission systems, Microelectronics Reliability, 20 (1980) 91-100.
[7] R. Billinton, X. Tang, Selected considerations in utilizing Monte Carlo simulation in quantitative reliability evaluation of composite power systems, Electric Power Systems Research, 69 (2004) 205-211.
[8] C.L.T. Borges, An overview of reliability models and methods for distribution systems with renewable energy distributed generation, Renewable and Sustainable Energy Reviews, 16 (2012) 4008-4015.
[9] C.L.T. Borges, D.M. Falcão, Optimal distributed generation allocation for reliability, losses, and voltage improvement, International Journal of Electrical Power & Energy Systems, 28 (2006) 413-420.
[10] H.-C. Chen, Optimum capacity determination of stand-alone hybrid generation system considering cost and reliability, Applied Energy.
[11] D.P. Clancy, G. Gross, F.F. Wu, Probabilitic flows for reliability evaluation of multiarea power system interconnections, International Journal of Electrical Power & Energy Systems, 5 (1983) 101-114.
[12] S. Fockens, A.J.M. van Wijk, W.C. Turkenburg, C. Singh, Reliability analysis of generating systems including intermittent sources, International Journal of Electrical Power & Energy Systems, 14 (1992) 2-8.
[13] S. Fockens, A.J.M. van Wijk, W.C. Turkenburg, C. Singh, Application of energy based indices in generating system reliability analysis, International Journal of Electrical Power & Energy Systems, 16 (1994) 311-319.
[14] H. Haroonabadi, M.R. Haghifam, Generation reliability assessment in power markets using Monte Carlo simulation and soft computing, Applied Soft Computing, 11 (2011) 5292-5298.
[15] Y.-Y. Hong, L.-H. Lee, Reliability assessment of generation and transmission systems using fault-tree analysis, Energy Conversion and Management, 50 (2009) 2810-2817.
[16] A. Jain, S.C. Tripathy, R. Balasubramanian, Reliability and economic analysis of a power generation system including a photovoltaic system, Energy Conversion and Management, 36 (1995) 183-189.
[17] D. Kottick, M. Blau, Y. Frank, Reliability of supply to consumers as a function of the

installed generation reserve margin, Electric Power Systems Research, 33 (1995) 63-67.
[18] S.R. Lakshmi, S.C. Tripathy, K.S.P. Rao, R. Balasubramianian, Evaluation of generation system reliability indices by fast transform techniques, International Journal of Electrical Power & Energy Systems, 17 (1995) 281-287.
[19] T. Lekane, J. Gheury, J. Van Hecke, Assessing the impact of operating constraints and operating policy on the reliability level of a generation system, International Transactions in Operational Research, 3 (1996) 271-279.
[20] Y.-F. Li, E. Zio, A multi-state model for the reliability assessment of a distributed generation system via universal generating function, Reliability Engineering & System Safety, 106 (2012) 28-36.
[21] S. Narasimhan, S. Asgarpoor, A fuzzy-based approach for generation system reliability evaluation, Electric Power Systems Research, 53 (2000) 133-138.
[22] P. Pelacchi, D. Poli, The influence of wind generation on power system reliability and the possible use of hydrogen storages, Electric Power Systems Research, 80 (2010) 249-255.
[23] M.V.F. Pereira, S.H.F. Cunha, G.C. Oliveira, Application of reliability evaluation methods to the planning of large hydroelectric systems, International Journal of Electrical Power & Energy Systems, 10 (1988) 142-150.
[24] O. Salmela, The effect of introducing increased-reliability-risk electronic components into 3rd generation telecommunications systems, Reliability Engineering & System Safety, 89 (2005) 208-218.
[25] W.-H. Shi, J. Chen, Reliability Assessment of Interconnected Generation Systems Based on Hourly Wind Speed Probability Model, Energy Procedia, 12 (2011) 819-827.
[26] C. Singh, N.V. Gubbala, An alternative approach to rounding off generation models in power system reliability evaluation, Electric Power Systems Research, 36 (1996) 37-44.
[27] C. Singh, N.V. Gubbala, Comparison of unit addition and fast Fourier transform methods for generation system reliability evaluation, International Journal of Electrical Power & Energy Systems, 18 (1996) 203-205.
[28] R. Sundaramurthi, C. Smidts, Human reliability modeling for the Next Generation System Code, Annals of Nuclear Energy.
[29] A.K. Verma, A. Srividya, B.C. Deka, Impact of a FACTS controller on reliability of composite power generation and transmission system, Electric Power Systems Research, 72 (2004) 125-130.
[30] Z.A. Yamayee, F.S. Ma, Effect of size and location of conventional and intermittent generation on system reliability, International Journal of Electrical Power & Energy Systems, 5 (1983) 94-100.
[31] X. Zhang, Z. Bie, G. Li, Reliability Assessment of Distribution Networks with Distributed Generations using Monte Carlo Method, Energy Procedia, 12 (2011) 278-286.
[32] R. Billinton, E. Khan, A security based approach to composite power system reliability evaluation, Power Systems, IEEE Transactions on, 7 (1992) 65-72.
[33] O. Alizadeh Mousavi, R. Cherkaoui, M. Bozorg, Blackouts risk evaluation by Monte Carlo Simulation regarding cascading outages and system frequency deviation, Electric Power Systems Research, 89 (2012) 157-164.

09/04/2012

The Objective Structured Clinical Examination: A study on satisfaction of students, faculty members, and tutors

Marzieh Amiri [1], Mansoureh Nickbakht [2]

[1.] Lecturer, Musculoskeletal Rehabilitation Research Center, Department of Audiology, Faculty of Rehabilitation Sciences, Ahvaz Jundishapur Universitry of Medical Sciences, Ahvaz, Iran
[1.] Msc. In Rehabilitation Administration. Musculoskeletal Rehabilitation Research Center, Faculty of Rehabilitation Sciences, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
nikbakht@ajums.ac.ir

Abstract: Introduction: Objective structured clinical examination (OSCE) is a valid and reliable instrument for effectively assessing medical students' training activities. Students' and examiners' satisfaction is an important part of the process. The purpose of this study was to investigate students' and examiners' opinions about the audiology exam in Ahvaz Jundishapur University of Medical Science. **Methods:** Fifty-two people, including students and faculty members in the field of audiology and instructors, participated in the survey. The test was divided into 10 sections, with five-10 minutes allocated for each test section. Participants were asked their opinion about the facilities and equipment used in the test section, the physical environment for the test, the answer sheet in each section, standardized patients, selected patients, time allocated for each section, and the examiners' method. **Results:** The majority of students and examiners preferred OSCE to conventional methods. Highest satisfaction in both groups was related to the equipment, and the lowest satisfaction was related to the time allotted to each test section.
Conclusion: It is better to use the OSCE method than conventional method to evaluate the end-of-semester training course. The time allocated to each test section should be increased.
[Marzieh Amiri, Mansoureh Nickbakht. **The Objective Structured Clinical Examination: A study on satisfaction of students, faculty members, and tutors**. *Life Sci J* 2012;9(4):4909-4911] (ISSN:1097-8135). http://www.lifesciencesite.com. 737

Keywords: Educational Measurement; Clinical Competence; Medical Students

1. Introduction

Currently, methods of assessing students examine knowledge and competence (1). Objective Structured Clinical Examination (OSCE) is a performance-based test (2) and a strategy for evaluating communication skills, critical thinking, problem-solving ability, practical skills, and the decision making (3). Studies have shown that a compared to conventional evaluation method, OSCE is a better tool (4).

Teaching clinical skills at the international level is growing, but written tests assess only students' cognitive ability and cannot evaluate students' actual behavior in clinical settings (5). OSCE is one of the best methods for student evaluation. Student and examiner both play an important role in conducting a good OSCE test, so it is important to satisfy both groups. In this test, a sense of injustice and resentment of the practical test is reduced. In Bolhari' study, all examiners believed OSCE sections can measure the student's skills, while only 43 percent believed that the section can measure the student's knowledge (6). In a satisfaction survey conducted by Hatala, et al., the majority of students believed that a sense of patient continuity is created in this test (7). In this study, we tried to survey the audiology students' opinions of Ahvaz Jundishapur University of Medical Sciences on an OSCE test.

2. Material and Methods

2.1. Context

At the end of each semester, clinical skills of audiology students are assessed. This evaluation was conducted in the traditional way for many years, but since early 2012, the OSCE method has been used. The test for these individuals includes the 10 section (case history, audiologic interpretation, otoscopy, immittance audiometry, pure tone audiometry, masking, tuning fork tests, site of lesion tests, audiogram interpretation, decision-making, and consultation). Time duration of each section is between five and 10 minutes. Testers were faculty and instructors in the audiology department.

2.2. Participants

All third and fourth-year audiology students in Ahvaz Jundishapur University of Medical Sciences (43 students) and members from the audiology department (9 persons) participated in the survey.

2.3. Instrument

The tool was a questionnaire that assesses the satisfaction about facilities and equipment used in the test station, physical environment of the test, answer sheets for each section, standardized patients,

introduced diseases, time allocated for each section, and methods of monitoring the examiners in each section. The participants were asked to express their views in a five-point Likert scale for each item with a range of 1-5 for very low to very high satisfaction. At the end of the questionnaire, participants were asked to respond to a Yes/No question to indicate whether OSCE test is a better alternative than the traditional method.

3. Results

Fifty-two questionnaires were completed by 43 students and nine examiners. All examiners were highly satisfied with the condition of the equipment and diseases listed in each section while only 26 students (60.5 percent) said they were highly satisfied with the equipment. Most dissatisfaction in both groups was related to the duration of each section.

Table1. Satisfaction of OSCE items in examiners and students

Items	n=9 (examiners)			n=43 (students)			n=52 (total respondents)		
	Level of satisfaction			Level of satisfaction			Level of satisfaction		
	Very high and High	Moderate	Low and Very low	Very high and High	Moderate	Low and Very low	Very high and High	Moderate	Low and Very low
	N (%)								
Equipment	9(100)	0	0	26(60.5)	8(18.6)	9(20.9)	35(67.3)	8(15.4)	9(17.3)
Physical environment	5(55.56)	4(44.44)	0	19(44.2)	12(27.9)	12(27.9)	24(46.2)	16(30.8)	12(23)
Answer sheets	8(88.89)	1(11.11)	0	20(46.5)	12(27.9)	11(25.6)	28(53.8)	13(25)	11(21.2)
Standardized patient	7(77.78)	2(22.22)	0	22(51.2)	12(27.9)	8(18.6)	29(55.8)	14(26.9)	8(15.4)
Introduced diseases	9(100)	0	0	15(34.9)	18(41.9)	10(23.3)	24(46.2)	18(34.6)	10(19.2)
Time duration in stations	5(55.56)	3(33.33)	1(11.11)	8(18.6)	6(14)	29(67.4)	15(28.8)	7(13.5)	30(57.7)
Manners of monitoring and the examiners	8(88.89)	1(11.11)	0	24(55.8)	12(27.9)	7(16.3)	32(61.5)	13(25)	7(13.5)

Results of the satisfaction survey are shown in Table 1. Participants in the survey were asked whether the clinical exam using OSCE style is better than a traditional style, which had been used in the previous semester. Sixty-five percent of students (28 students) assessed OSCE better than traditional tests, which had been used in their previous semesters, while the rate for examiners was 88 percent (eight people).

Fisher's exact test and Chi-square test were used to examine the differences between students' and examiners' views. There was a significant difference between the views of these two groups on satisfaction with the test equipment, the questionnaire forms, the section length, the introduced diseases in each section, and examiners assessed these items higher than students at a significance level of 0.05.

A comparison of the views of third and fourth-year students found no significant differences between the views of these two groups on six items in the survey, and the only significant difference was observed in the level of satisfaction with introduced diseases in the sections, and third-year students were more satisfied with the diseases listed in this test ($p = 0.024$).

4. Discussions

The main objective of the study was to examine students' and audiology members' satisfaction level of the OSCE test. Most participants in the study were satisfied with the test, and they preferred this test to traditional appraisals. Of course, the examiners generally had more positive views than the students about the various items on the test. In Erfanian and Khadivzadeh' study, 80 percent of midwifery students of Mashhad University of Medical Sciences (Iran) said that they are highly and very highly satisfied with the OSCE test. The OSCE had 10 sections for 62 students (8). In the Kurz survey, nursing students and faculty declared their high satisfaction with the OSCE format (9). Huang, et al., asked medical students to rate the OSCE in a five-point Likert scale. Eighty percent of them selected satisfied and very satisfied instead of dissatisfied, very dissatisfied, and neutral. Satisfaction was for all aspects of OSCE, including exam content, exam atmosphere, and improvement in clinical skills after the exam, standardized Patient performance, and overall satisfaction with the exam. In Huang's study, students had little satisfaction with the test environment due to noise interference in other sections of the test (10). This option in our

study earned 55 percent satisfaction from examiners and 44 percent satisfaction from students.

In our study, the highest level of satisfaction among audiology students was with the equipment used in OSCE (60 percent). Satisfactions with the manner of supervision and examiners, and also with the standardized patient satisfaction were 55 percent and 51 percent, respectively. However, the majority of students were dissatisfied with the allotted time for each section, and only 18 percent of them assessed the time as appropriate while 55 percent of the examiners stated their satisfaction with the allotted time. It seems that due to disagreement between examiners and students on the test equipment, response forms, time duration in the section, and diseases introduced in each section, it is necessary to conduct a review of these items to achieve students' satisfaction.

Acknowledgements:

This research (No. pht 9126) was conducted with sponsorship and support of the vice chancellor for developing research and technology, Ahvaz Jundishapur University of Medical Sciences. We acknowledge the Research Consultation Center (RCC) for technical assistance.

Corresponding Author:
Mansoureh Nickbakht,
Musculoskeletal Rehabilitation Research Center,
Faculty of Rehabilitation Sciences,
Ahvaz Jundishapur University of Medical Sciences,
Ahvaz, Iran
Tel: +98.9163070953
E-mail: nikbakht@ajums.ac.ir

References

1. Lindblom-Ylanne S, Lonka K, Leskinen E. On the predictive value of entry-level skills for successful studying in medical school. Higher education, 1999. 37, 239–258.
2. McWilliam P.L, Botwinski C.A. Identifying strengths and weaknesses in the utilization of Objective Structured Clinical Examination (OSCE) in a nursing program. Nursing education perspectives, 2012. 33(1),35-9.
3. Silva C.C, Lunardi, A. C, Mendes F. A, Souza, F. F, Carvalho, C. R. Objective structured clinical evaluation as an assessment method for undergraduate chest physical therapy students: a cross-sectional study. REVISTA BRASILEIRA DE FISIOTERAPIA, 2011. 15(6), 481-6.
4. Mondal R, Sarkar S, Nandi M, Hazra A. Comparative Analysis Between Objective Structured Clinical Examination (OSCE) and Conventional Examination (CE) As a Formative Evaluation Tool in Pediatrics in Semester Examination for Final MBBS Students. Kathmandu University medical journal (KUMJ), 2012. 10(37),62-5.
5. El-Khoury L.H, Saab B, Musharrafieh U, Antoun J. Impact of a 3-day OSCE Workshop on Iraqi Physicians. FAMILY MEDICINE, 2012. 44(9), 627-32.
6. Bolhari J. Editorial.Iranian Journal of Psychiatry and Clinical Psychology, 2007. 13(1), 3-4.
7. Hatala R, Issenberg S. B, Kassen B. O, Cole G, Bacchus C. M, Scalese R. J. Modification of an OSCE format to enhance patient continuity in a high-stakes assessment of clinical performance. BMC Medical Education, 2011.11(23). Doi: 10.1186/1472-6920-11-23
8. Erfanian F, Khadivzadeh T. Evaluation of midwifery students' competency in providing intrauterine device services using objective structured clinical examination. Iranian journal of nursing and midwifery research, 2011. 16(3), 191-6.
9. Kurz J.M, Mahoney K, Martin-Plank L, Lidicker J. Objective structured clinical examination and advanced practice nursing students. Journal of professional nursing, 2009. 25(3), 186-91.
10. Huang Y.S, Liu M, Huang C. H, Liu K. M. Implementation of an OSCE at Kaohsiung Medical University. The Kaohsiung journal of medical sciences, 2007. 23(4),161-9.

12/12/2012

Preparation and Characterization of Conducting Polystyrene Graft Polyaniline

Seyyed Hossein Hosseini[1]* and Seyed Mahdi Musavi[2]

[1]Department of Chemistry, Faculty of Science, Islamic Azad University, Islamshahr branch, Tehran-Iran

[2]Department of Chemistry, Faculty of Science, Islamic Azad University, Shahr-e-Ray branch, Tehran-Iran

***Corresponding Author**: Seyed Hossein Hosseini, Department of Chemistry, Faculty of Science, Islamic Azad University, Islamshahr branch, Tehran-Iran; Email: shhosseini@iiau.ac.ir, Tel./Fax: +98-21-77312543, (+989121374816).

Abstract: The electroactive graft of polystyrene (PS) and polyaniline (PANi) copolymer can be synthesized by chemical and electrochemical polymerization method using a polymer as precursor which contains an aniline moiety in its side chain. Poly(styrene-*co*-p-chloromethylstyrene), poly(S-*co*-PCMS), was chemically synthesized with chloromethylated styrene then copolymerized styrene by free radical mechanism. Poly(styrene-*co*-p-aminoanilinemethylstyrene), poly(S-*co*-PAAMS), was prepared chemically with poly(S-*co*-PCMS) and 1,4-phenylenediamine, too. Therefore, PANi grafted to PS, (PS-*g*-PANi) synthesized by ammonium peroxydisulfate as the oxidant and p-toluenesulfonic acid in dimethylsulfoxide (DMSO) solution with adding aniline on oxidated poly(S-*co*-PCMS). The formation of graft copolymer was confirmed by FT-IR, UV-visible, ^{1}H and ^{13}C-FT-NMR spectroscopy. Therefore, molecular weight and amount of PANi grafted on PS have been measured by gel permeation chromatography and elemental analysis, respectively. The thermal analysis of graft copolymer was carried out using thermogravimetic analysis (TGA) and differential scanning calorimetric (DSC). The solution of poly(S-*co*-PAAMS) in DMSO was spin-coated on GC electrode and electrochemically polymerized in electrolytic mixture solution consisting of 0.05 M aniline, 0.1 M H_2SO_4 and 1 M HCl. Electrolysis constant potential showed that aniline groups in the precursor were oxidized to form PANi, that is, they acted as grafting centers at which the PANi grew. Results of scanning electron microscopy (SEM) results and conductivity measurements supported the formation of the graft copolymer. The morphological feature of PS-*g*-PANi copolymer film has been shown homogeneous structure, the PS/PANi composite film shows irregular structure. Electrical conductivity of copolymer has been studied by four probe method and produced 3.3×10^{-2} S/cm.

[Seyyed Hossein Hosseini and Seyed Mahdi Musavi. **Preparation and Characterization of Conducting Polystyrene Graft Polyaniline.** *Life Sci J* 2012;9(4):4912-4925] (ISSN:1097-8135). http://www.lifesciencesite.com. 738

Key word: *graft copolymers, polyaniline, polystyrene*

INTRODUCTION

Conjugated polymers maybe made by a variety of techniques, including cationic, anionic, radical chain growth, co-ordination polymerization, step growth polymerization or electrochemical polymerization. Electrochemical polymerization occurs by suitable monomers which are electrochemically oxidized to create an active monomeric and dimeric species which react to form a conjugated polymer backbone [1]. The main problem with electrically conductive plastics

stems from the very property that gives it its conductivity, namely the conjugated backbone. This causes many these polymers to be intractable, insoluble films or powders that cannot melt [2]. There are two main strategies to overcoming these problems. There are to either modify the polymer so that it maybe more easily processed, or to manufacture the polymer in its desired shape and form. There are, at this time, four main methods used to achieve these aims [3].

The first method is to manufacture a malleable polymer that can be easily converted into a conjugated polymer. This has been done when the initial polymer is in the desired form and then, after conversion, is treated so that it becomes a conductor. The treatment used is most often thermal treatment. The precursor polymer used is often made to produce highly aligned polymer chain which are retained upon conversion. These are used for highly orientated thin films and fibers. Such as these films and fibers are highly anisotropic, with maximum conductivity along the stretch direction [4].

The second method is the synthesis of copolymers or derivatives of a parent conjugated polymer with more desirable properties. This method is the more traditional one for making improvements to a polymer. What has been done is to try to modify the structure of the polymer to increase its processibility without compromising, its conductivity or its optical properties. All attempts to do this on polyacetylene have failed as they always significantly reduced, its conductivity. However, such this attempt on polythiophenes and polypyrroles proved more fruitful. The hydrogen on carbon 3 on the thiophene or the pyrrole ring was replaced with an alkyl group with at least four carbon atoms in it. The resulting polymer, when doped, has a comparable conductivity to its parent polymer whilst be able to melt and it is soluble. A water soluble version of these polymers has been produced by placing carboxylic acid group or sulphonic acid group on the alkyl chains. Then such system can maintain charge neutrality in its oxidized state and so they effectively dope themselves. Such polymers are referred to as "self-doped" polymers. One of the most highly conductive derivative of polythiophene is made by replacing the hydrogen on carbon three with a $-CH_2-O-CH_2CH_2-O-CH_2CH_2-O-CH_3$. This is soluble and reaches a conductivity of about 1000 S cm^{-1} upon doping [5].

The third method is to grow the polymer into its desired shape and form. An insulating polymer impregnated with a catalyst system is fabricated into its desired form. This is then exposed to the monomer, usually a gas or a vapour. The monomer then polymerizes on the surface of the insulating plastic producing a thin film or a fibre. This is then doped in the usual manner. A variation of this technique is electrochemical polymerization with the conducting polymer being deposited on an electrode either the polymerization stage or before the electrochemical polymerization. This maybe used for further processing of the conducting polymer. For instance, by stretching aligned bends of polyacetylene/polybutadiene the conductivity increase 10 fold, due to the higher state of order produced by this deformation [6].

The final method is the use of Langmuir-Blodgett trough to manipulate the surface active molecules into a highly ordered thin films whose structure and thickness which are controllable at the molecular layer. Amphiphilic molecules with hydrophilic and hydrophobic groups produces monolayers at the air-water surface interface of a Langmuir-Blodgett trough. This is then transferred to a substrate creating a multilayer structure comprised of molecular stacks which are normal about 2.5 nm thick. This is a development from the creation of insulating films by the same technique. The main advantage of this technique is its unique ability to allow control over the molecular architecture of the conducting films produced. It can be used to create complex multilayer structures of functionally different molecular layers as determined by the chemist. By producing alternating layers of conductor and insulator it is possible to produce highly anisotropic film which is conducting within the plane of the film, but insulating across it [7]. In this investigation, we pointed out the production of graft copolymer films of polystyrene (PS) and polyaniline (PANi) (Scheme I). Emeraldine salt (half oxidized PANi, y=0.5) is the most

conductive form of PANi, which can be obtained by protonation of emeraldine base and oxidation of leucoemeraldine base as shown in Figure 1.

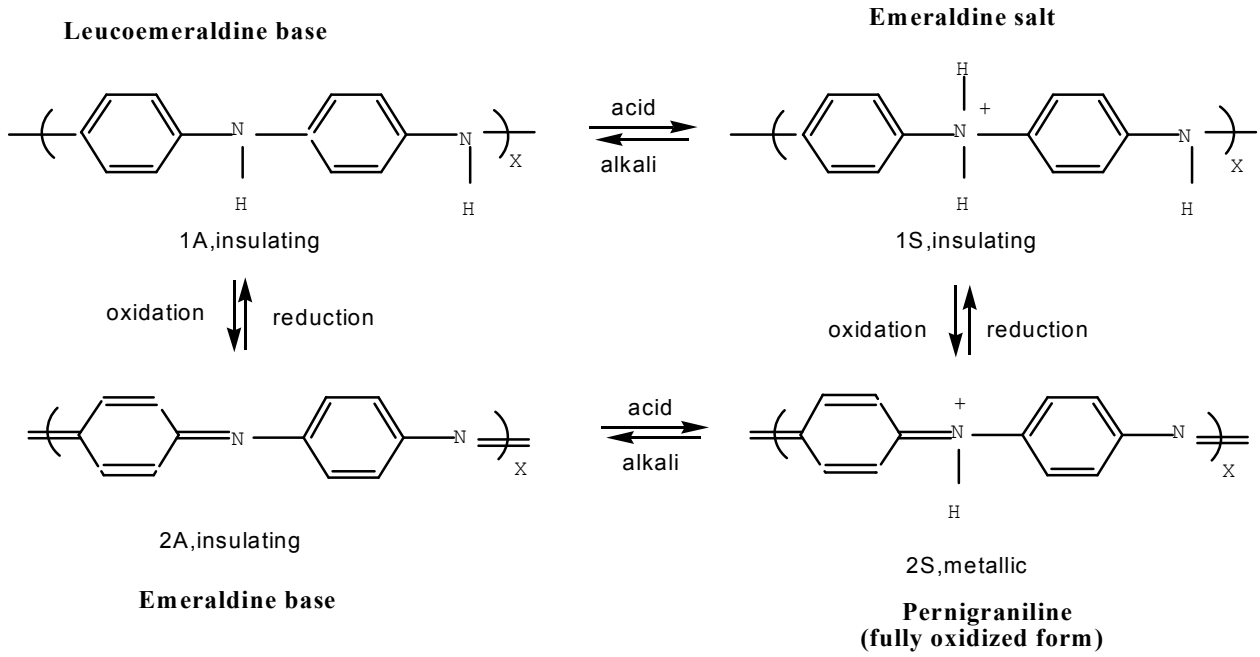
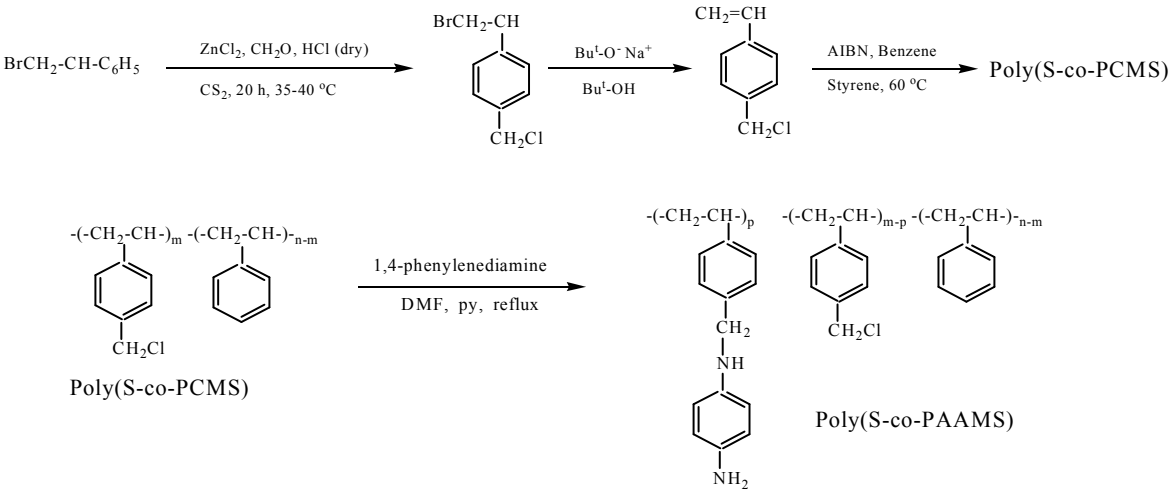
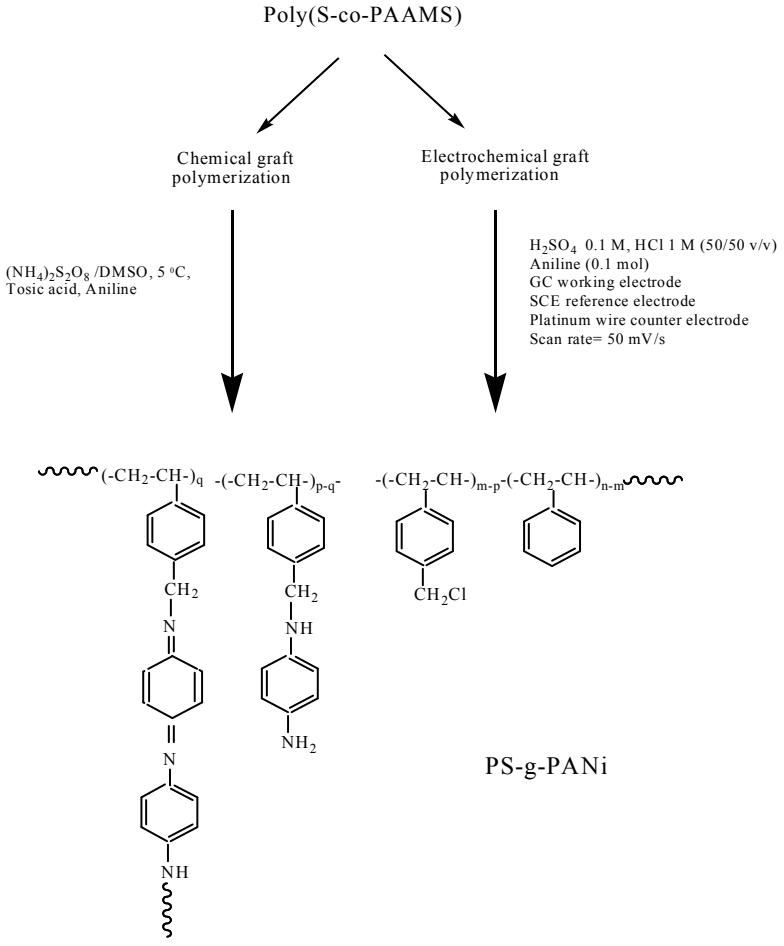


Figure 1. The different of polyaniline forms


BrCH2-CH-C6H5
ZnCl2, CH2O, HCl (dry)
CS2, 20 h, 35-40 °C
BrCH2-CH
CH2Cl
But-O- Na+
But-OH
CH2=CH
CH2Cl
AIBN, Benzene
Styrene, 60 °C
Poly(S-co-PCMS)
-(-CH2-CH-)m -(-CH2-CH-)-n-m
CH2Cl
Poly(S-co-PCMS)
1,4-phenylenediamine
DMF, py, reflux
-(-CH2-CH-)p
-(-CH2-CH-)m-p -(-CH2-CH-)-n-m
CH2
NH
NH2
CH2Cl
Poly(S-co-PAAMS)


Poly(S-co-PAAMS)
Chemical graft polymerization
Electrochemical graft polymerization
(NH4)2S2O8 /DMSO, 5 °C,
Tosic acid, Aniline
H2SO4 0.1 M, HCl 1 M (50/50 v/v)
Aniline (0.1 mol)
GC working electrode
SCE reference electrode
Platinum wire counter electrode
Scan rate= 50 mV/s
(-CH2-CH-)q
-(-CH2-CH-)p-q-
-(-CH2-CH-)m-p-(-CH2-CH-)n-m
CH2
N
N
NH
NH2
CH2Cl
PS-g-PANi

Scheme I

The preceding works, we have prepared graft copolymer polypyrrole on poly(vinyl acetate), PS [8,9] and polyaniline on polyacrylonitrile [10]. These These copolymer showed high conductivity but decreased solubility by increasing polypyrrole ratio on copolymers.

Here we determined the composition as well as the conductivity and solubility properties of those graft copolymers PS-*g*-PANi depending on the reaction conditions.

The graft copolymer materials prepared from hydrophilic polymers, PS-*g*-PANi could be appropriate for various applications in science and technology due to the good mechanical properties and their electrical conductivity. The changes and maximum balance's of doping are limited. But they have stability of electrical conduction and high physical resistance.

EXPERIMENTAL

Materials

Aniline (GPR) was dried with NaOH, fractionally distillated under reduced pressure from sodium or CaH_2. Polystyrene with 5% polybutadiene (Aldrich, Medium molecular weight) was used as matrix polymer. Acetonitrile (Merck) was dried on silicagel, distillated on P_2O_5 in the presence of nitrogen gas. 1,4-Phenylenediamine (Aldrich) purified by recrystalled in ethanol. Azobisisobutyronitrile (AIBN) and all the other materials and gases used in this work were purchased from Merck chemicals and purified, or were prepared by literature methods.

Instrumentals

Elemental analysis was determined with Perkin-Elmer 2400 CHN. A fourier-transform infrared spectrometer (8101 M-Shimadzu) was used in spectral measurements of the polymer and reported by sharp (sh), weak (w), broad (b), middle (m). Cyclic voltammetry and electrochemical polymerization were carried out using digital potentiostate DP8 and the electrical conductivity of polymer was measured at room temperature by the four-probe technique (ASTM Standards, F 43-93). Proton nuclear magnetic resonance (FT-H^1, ^{13}C-NMR) spectra were recorded at 250 MHz on a Bruker WP 200 SY spectrometer . NMR data are reported in the following order: chemical shift (ppm), spin multiplicity (s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, b= broad) and integration. Visible spectra were obtained by Perkin Elmer Lambda 15 spectrophotometer. Molecular weights were measured at 30 ^{0}C with a gel permeation chromatography (GPC) (Waters Associates, model 150-C). Three styragel packed columns with different pore sizes (10^4-10^6 A^0) were used. The mobile phase was tetrahydrofuran (THF) with flow rate of 1.5 ml/min. The solution concentration was 0.2 wt%. Calibration of the instrument was performed with nine standard samples of monodisperse polystyrene having molecular weights between 3.0×10^3 and 1.4×10^6. The thermal properties of polymer were studied by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC) of PL Thermal Science. Scanning electron microscopy (SEM) was employed to study the type of surface morphology of polymer. A Cambridge S-360 SEM was used for this purpose.

Synthesis of p-(2-bromoethyl)benzyl chloride

2-Phenylethyl bromide (70 g, 0.38 mole) was dissolved in 50 ml of carbon disulfide in a 1 liter three-necked round-bottomed flask equipped with a stirrer, gas inlet tube, and a reflux condenser. Anhydrous zinc chloride (11 g) and paraformaldehyde (13 g) were added to the flask in three separate portion during the course of reaction. Hydrogen chloride was bubbled into the reaction mixture the rapid stirring for 20 hr at 35-40 ^{o}C. The lower phase of the reaction mixture was soluble in water. The organic layer was washed with water, diluted sodium carbonate and again with water. After being dried with anhydrous sodium sulfate, the carbon disulfide was distilled from the product and unreacted 2-phenylethyl bromide was recovered by distillation at a pressure of about 10 Torr. The product a mixture of o- and p-(2-bromoethyl)benzyl chloride was obtained by distillation bp 91-94 ^{o}C/2 Torr. The mixture was dissolved in 200 ml of petroleum ether and the solution was chilled overnight in a refrigerator. The remaining liquid was decanted and obtained solid was

recrystallized twice from 200 ml of petroleum ether. The yield was 39 g (54%); mp 48-50 ^{o}C. [10] Gas chromatography of obtained compound showed one peak (Carbowax 20M, 10%, Celite 545, 1 m, 160 C, Hz). The ^{1}H-NMR spectrum showed 2.97-3.64 (-CH_2CH_2Br), 4.47 (-CH_2Cl) and 7.19 ppm (aromatic protons).

Synthesis of p-chloromethylstyrene

A portion of sodium metal 0.32 g (0.014 mol) was carefully added to 25 ml of tert-butyl alcohol at 50 ^{o}C. After all the metal reacted, the solution was cooled to the room temperature and 3 g (0.013 mole) of p-(2-bromoethyl)benzyl chloride was added to the solution. The mixture was stirred at 35 ^{o}C for 2 hr. Then the reaction mixture was poured into 500 ml of water and extracted with ether then extraction dried with anhydrous sodium sulfate. The ether was removed under reduced pressure and the residue was distilled in the presence of p-tert-butyl catecohol to give 1.7 g of p-chloromethylstyrene, bp 88-90 ^{o}C/ 2 Torr. [11]

Preparation of poly(styrene-co-p-chloromethylstyrene), poly(S-co-PCMS)

The copolymerization of p-chloromethylstyrene and styrene was carried out in a sealed tube at 60 ^{o}C. The required amounts of p-chloromethylstyrene, styrene, AIBN, and then benzene as solvent were charged into a pyrex glass tube, which was then degassed under vacuum by conventional freezing and thawing technique and sealed off under vacuum. All copolymerizations were poured into a large amount of methanol to precipitate the copolymer. The resulting copolymers were then purified by the reprecipitation of the benzene solution of with excess methanol. The composition of the copolymer was calculated from their elementary analysis of cholorine, 19.8% (2.77 mmol of Cl/g).

UV (DMSO): λ_{max} = 221 nm (3.1 intensity), 270 nm (0.7 intensity).

FT-IR: 3009(m), 2980(m), 1610(m), 1550 (b), 1490(sh), 1450(sh),1360(sh), 1090(b), 1020(b), 760(w), 690(sh) and 550(w) cm^{-1}.

^{1}H-NMR ($CDCl_3$) δ 6.5-7.5 (b and d, 6H), 3.36 (s, 2H), 1.80 (b and m, 1H), 1.43 (b, 2H) ppm.

^{13}C-NMR ($CDCl_3$) δ 143, 127, 126, 125, 68.1, 40.5, 30.1 ppm.

Preparation of poly(styrene-co-p-aminoanilinemethylstyrene), poly(S-co-PAAMS)

A 250 ml three-necked flask containing 30 ml dimethylformamide(DMF) and 1.5 g poly(S-*co*-PCMS) is equipped with a mechanical stirrer and maintained at temperature of 75 ^{o}C by an external water bath. Then 1 g (0.01 mol) 1,4-phenylenediamine, 2 g anhydrous pulverized sodium carbonate and 5 ml pyridine are added to the flask and the resulting mixture is stirred and heated for 3 hours. At the end of this time, the polymer solution is filtered and precipitate into ice methanol; the product washed with methanol and dried under vacuum.

UV (DMSO): λ_{max} = 230 nm (3.2 intensity), 282 nm (1.7 intensity), 325 nm (0.4 intensity).

FT-IR: 3400(b), 3010(w), 2980(w), 1610(m), 1570(b), 1550(m), 1475(m), 1445(mh), 1363(w),1080(m), 1015(w), 760(w) and 680(m) cm^{-1}.

^{1}H-NMR (d_6-DMSO) δ 6.5-7.5 (b and d, 8H), 3.35 (b, 3H), 1.78 (b, 1H), 1.40 (b, 2H) ppm.

^{13}C-NMR (d_6-DMSO) δ 145, 128, 127, 125, 96.4, 94.5, 67.4, 40.4, 29.3, 26.5 ppm.

Preparation of polystyrene-graft-polyaniline, PS-g-PANi

0.5 g poly(S-*co*-PAAMS) is completely dissolved in 30 mL of dimethylsulfoxide (DMSO). 1 g aniline and 0.5 g p-toluensulphonic acid are added to the solution, then mixture is vigorously stirred and reduced temperature to 5 ^{o}C. In a separate container 2 g (0.0088 mmol) $(NH_4)_2S_2O_8$ (ammonium peroxydisulphate) is dissolved in 10 mL tosic acid solution. The oxidant solution is slowly added at a rate of approximately 5 mL per minute to the mixture.

After the mixture is allowed to stir for 10 minutes, a solution containing 1 g sodium sulfite in 5 mL water/DMSO is added to the mixture. The mixture is stirred for about 2 hours and polymer solution is filtered using G2 sintered glass filter, then precipitated into ice methanol. The product was washed successively by distilled water and methanol and dried at 60 ^{o}C for 24 h.

UV (DMSO): λ_{max} = 230 (1.7 intensity), 292 nm (2.9 intensity), 330 nm (2.7 intensity), 640 nm (1.1 intensity).
FT-IR: 3335(b), 3175(m), 2985(m), 1733(w), 1593(m), 1516(m), 1329(m), 1275(m), 1181(sh), 1064(m), 1005(m), 880(s), 845(m), 693(w), 573(w) cm^{-1}.
^{1}H-NMR (d_6-DMSO) δ 6.0-7.5 (b, H-aromatics), 4.6-4.8 (-NH, $-NH_2$), 3.3-3.5 (-CH_2Cl and $-CH_2$), 1-2 (b, H-aliphatics) ppm.
^{13}C-NMR (d_6-DMSO) δ 146, 144, 142, 140, 122, 121, 117.1, 116.1, 97.5, 95.6, 68.5, 41.4, 30.7, 29.5, 27.1 ppm.

Electrochemical preparation of PS-g-PANi
Electrochemical synthesis of PS-*g*-PANi was carried out using a conventional three electrode system with a SCE reference, platinum wire counter electrode and GC disk working electrode. The solutions cyclic voltammetry are 0.1 M H_2SO_4 and 1 M HCl (50/50 v/v). Firstly coated a suitable amount poly(S-*co*-PAAMS) on the GC disk electrode using casting method and then add 0.05 M aniline. Polymer was grown on GC disk electrode under scan potential in the range of 0.3 to 1.4 mV and scan rate 50 mV/s. The thickness of the film was ca. 23-30 µm as determined by scanning electron microscopy.

RESULTS AND DISCUSSION
In order to assess the percentage of polymers in mixture we use elemental analysis. Assessing the percentage of the mixture is so important because in this way we are able to justify condition of electron conductivity in mixture. In all the mixtures, we study the variation of parameters such as: density of the oxidant, solvent effect, density of monomers and based on polymers. We are intended to get the acceptable condition in order to use the sensitivity usage. For this reason we studied elemental analysis on reliable films in view of electrical conductivity, stability and mechanical properties [8-10]. If each styrene ring had received one chloromethyl group the resulting product would have had a chloride content of 22.9%. Elemental analysis for poly(S-*co*-PCMS) showed 19.8% (2.77 mmol of Cl/g), which means that 85*%* of the rings were chloromethylated. Since provided polymer bases on aniline and on the other hands, PS is without nitrogen atom, so by measuring the percentage of nitrogen in elemental analysis, we can achieve the percentage of PANi in copolymer. To assess the aniline and PANi percentages in copolymer we use the following equations. In beginning we will measure percentage of theoretical mass of nitrogen in each monomer unit or base polymer poly (S-*co*-PAAMS) and then on PS-*g*-PANi.

$$\text{Percentage of theoretical mass of nitrogen in polymer} = \frac{\text{Molecular mass of nitrogen}}{\text{Mass unit of monomer (p-aminoanilinemethylstyrene)}} * 100$$

$$\text{Percentage of theoretical mass of nitrogen in poly(S-co-PAAMS)} = \frac{14}{224} * 100 = 6.25$$

$$\text{Percentage of aniline in copolymer poly(S-co-PAAMS) (\% aniline)} = \frac{\%N \text{ Found}}{\text{Theoretical mass of nitrogen in polymer}} * 100$$

$$(\%\text{ aniline}) = \frac{8.5}{6.25} * 100 = 136 \Longrightarrow \frac{136}{2} = 68$$

which means that 68% of the chloromethylated rings were converted phenylamine. On the other hands, for calculation of percentage of PANi on graft copolymer, we measured according following equations, we know percentage of nitrogen after grafting is 19.2, so:

$$\text{Percentage of aniline or PANi in PS-g-PANi (\% PANi)} = \frac{19.2}{6.25} * 100 = 307$$

which means that aniline would growth on poly(S-*co*-PAAMS) as well.

Figure 2 (a,b) shows FT^{1}H-NMR and FT^{13}C-NMR spectra of poly(S-*co*-PMMAS). Figure 3 shows FT^{1}H-NMR spectrum of PS-*g*-PANi. The occurrence of the peaks at 6-7.5 ppm indicates H-aromatics containing phenylenediamine and aniline groups. The peaks of 4.5-4.8 ppm and 3.3-3.7 ppm shows –NH, -NH_2 and –CH_2Cl, -CH_2- groups, respectively. Finaly, peaks at between 1-2.5 ppm indicate H-aliphatics for polymer backbone, too. The FT^{13}C-NMR spectrum of poly(S-*co*-PMMAS), Figure 2(b), shows peaks below 45 ppm and between 90-145 ppm for different types of aliphatic and aromatic carbons, respectively. The peak of 67.5 ppm can be related to the unreacted –CH_2Cl on main chain.

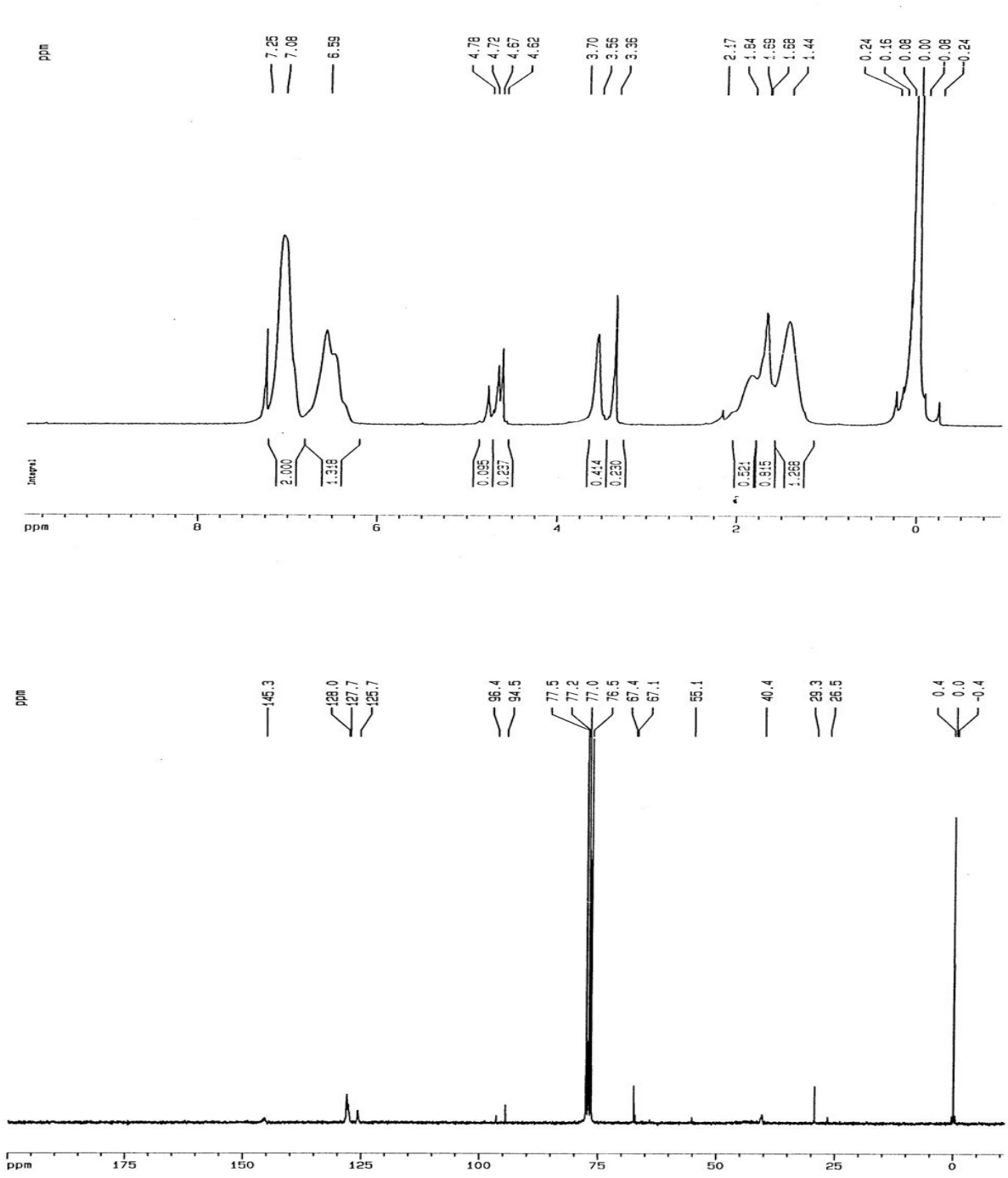


Figure 2. NMR Spectra of poly(S-*co*-PAAMS) a) FT ^{1}H-NMR b) FT ^{13}C-NMR

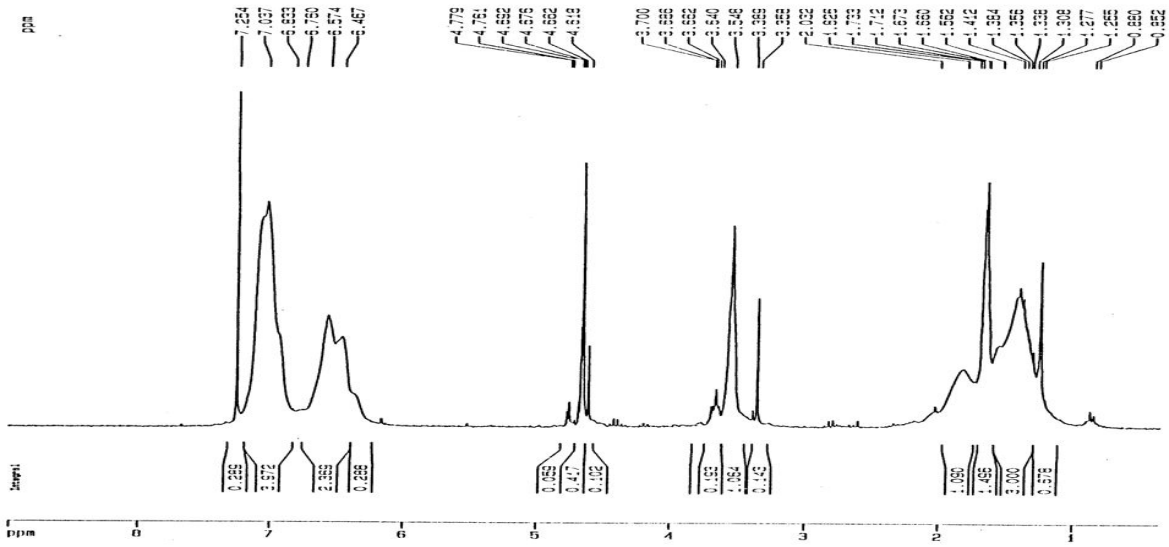


Figure 3. FT[1]H-NMR Spectra of PS-*g*-PANi

Electropolymerization of aniline on poly(S-*co*-PAAMS) carried out in the above condition. Figure 4 shows the cyclic voltammogram of PS-*g*-PANi formation on a Pt electrode that spin coated by poly(S-*co*-PAAMS) in aqueous, 1 M H_2SO_4, 0.1 M HCl and 0.1 M aniline solution (v = 50 mV/s); potential range between 0.3 to 1.4 V *vs*. SCE. The redox anodic and cathodic peaks appears in the initial curves and increase in intensity in each next cycles. At the same time, during the cycling, the electrode is covered with a thick deposit and extensive dissolution occurs. The solution near the electrode surface became an intensive green color. Figure 5 shows cyclic voltammograms of PS-*g*-PANi in different scan rate. The oxidation-reduction potentials of this copolymer is different with it's homopolymer. Therefore, cyclic voltammogram confirm that formation of copolymer. Spectroscopy data certify the formation of copolymer at the chemical polymerization, too. As it shows in voltmmogram, PS-*g*-PANi has reversibility and well stability on electrode's surface. The polymer obtained by electropolymerization was precipitated completely on an electrode surface. As it's shown in Figure 4 and 5, the oxidation of the polymer is done easily, but the reduction is done slowly. The growth of the polymer on the surface of the electrode has been done slowly, then in continue it becomes stable gradually.

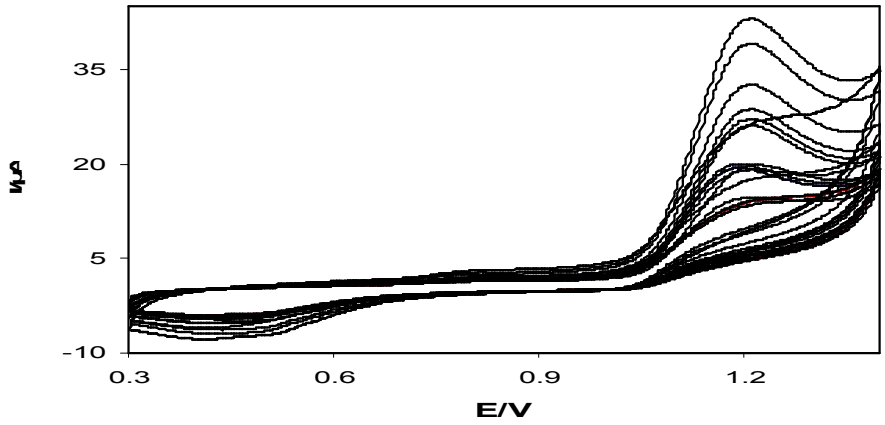


Figure 4. Cyclic voltammogram of PS-*g*-PANi formation on a Pt electrode that spin coated by poly(S-*co*-PAAMS) in aqueous 1 M H_2SO_4, 0.1 M HCl and 0.1 M aniline solution (v = 50 mV/s); potential range between 0.3 to 1.5 V *vs*. SCE (14 cycles).

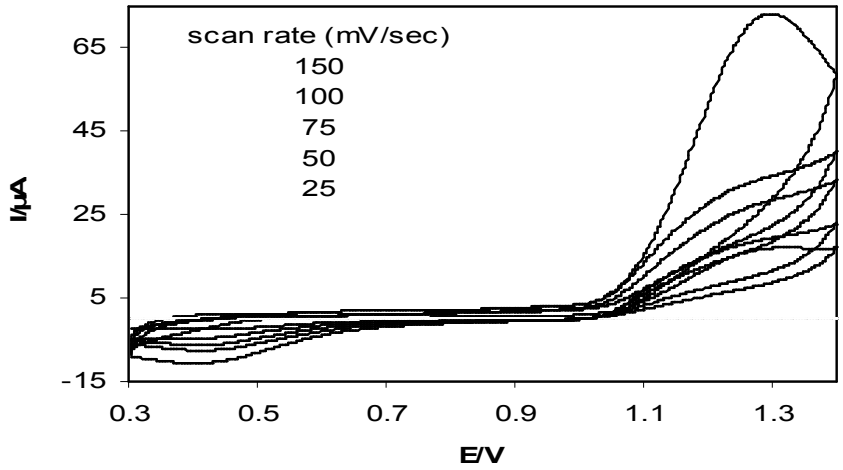


Figure 5. Cyclic voltammograms of PS-*g*-PANi on a Pt electrode in aqueous 1 M H_2SO_4, 0.1 M HCl and 0.1 M aniline solution (v = 50 mV/s); potential range between 0.3 to 1.4 V *vs*. SCE in different scan rates.

Figure 6 show the anodic (ipa) and cathodic (ipc) peak current via different scan rate (SCE reference electrode) of PS-*g*-PANi. The curves are linear, therefore, the polymers are stable on the electrode surface. Redox of reaction polymer film on electrode surface is a surface absorption type.

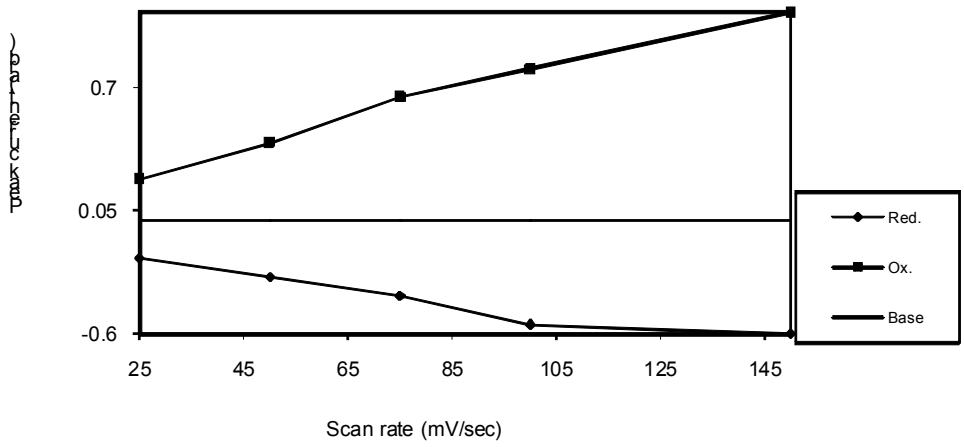


Figure 6. The anodic and cathodic peak current vs. different scan rates of PS-*g*-PANi.

The thermal properties of polymer were studied by thermogravimetric analysis (TGA), differential scanning calorimetry (DSC). Thermograms of STA (DSC and TGA) PS-*g*-PANi illustrated in Figure 7. According to these thermograms with STA thermograms of PS shows that softness, melting points and thermal resistance of the produced copolymer is higher than non-grafted copolymer.

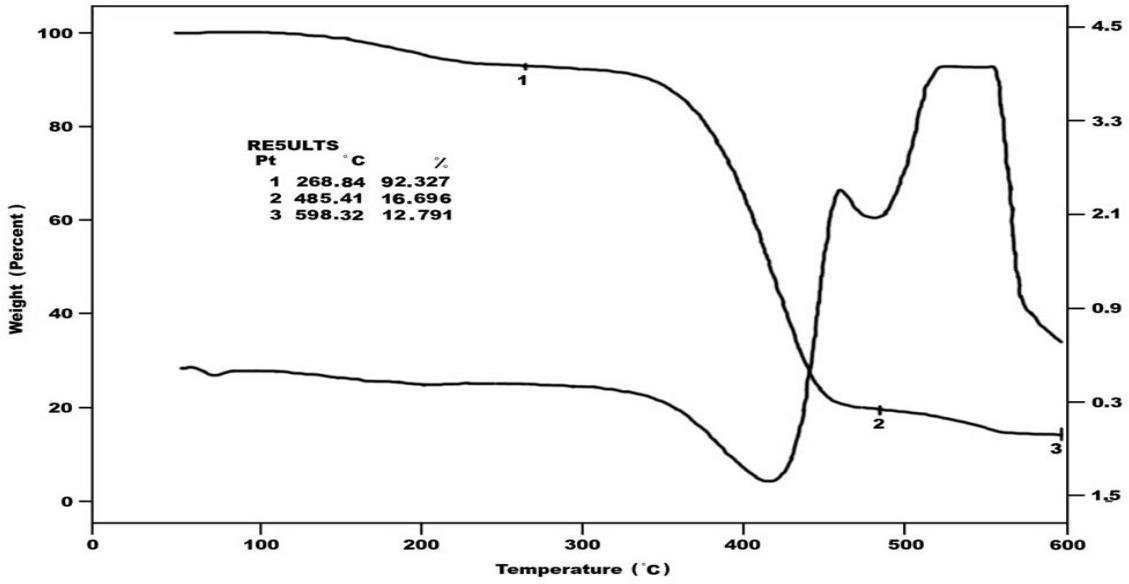


Figure 7. STA (TGA and DSC) Thermmograms of PS-*g*-PANi

As it shows in PS-g-PANi thermogram, this polymer in 50 ^{o}C started to become soften and up to 268.84 ^{o}C it approximately losses 7.628% of it's weight which is due to humidity and existing solvent or part of HCl in chains polymer, and in about 485.41 ^{o}C it will lose approximately degraded. PS-*g*-PANi is good stable to below 350 ^{o}C and in over 360 ^{o}C polymer starts it's complete distraction and continue to 450 ^{o}C. The methods of interpreting TGA results are numerous and also lack standardization. Some of these note the temperature of the 5-10% loss, or by the temperature inflection of the down ward slope, or by the first detectable break in the curve. The STA of PS-*g*-PANi illustrated that initial decomposition temperature (IDT), polymer decomposition temperature (PDT) and the maximum polymer decomposition temperature (PDT_{max}) are 350 ^{o}C, 400 ^{o}C and 480 ^{o}C, respectively. The residual weights (γ_c) of the polymers were reported at 600 ^{o}C that it is 12.791%. These polymers were stable in natural atmosphere up to presented temperatures.

Polymer structure and morphology are greatly affected by electrosynthetic conditions [12], such as electrode materials, solvent and electrolyte salts, oxygen and water content of the system and the current density used for electropolymerisation. Although a quantitative measurement of these effects has not been established, some general observations have been made. Thin films generally appear smooth, whilst thicker samples have a much more uneven textured surface [13]. Potentials used during electropolymerisation give rise to smoother films [14]. Scanning electron microscopy (SEM) of figures shows monotony, kind of phases and surface of polymers completely. SEM also shows that the doping/dedoping process is often accompanied by major changes in morphology [15], perhaps due to swelling caused by the insertion and removal of ions within the polymer matrix.

Figure 8 shows the SEM image of PS-*g*-PANi. As it shows in this figure monotony and single phase of polymers are completely clear. PANi shows a sponge-like morphology in contrast to PS-*g*-PANi, which have smooth surfaces indicating that no morphological characteristics could be observed. Earlier studies on the surface morphology of PS and poly(S-*co*-PCMS) have demonstrated theirs smooth surface. Despite the fact that PS-*g*-PANi contained about 307 wt% PANi and according to elemental analysis determination, no free PANi is seen in SEM picture of the graft copolymer. This observation enforces the assumption that covalent bonds between poly(S-*co*-PCMS) and PANi chain have been produced.

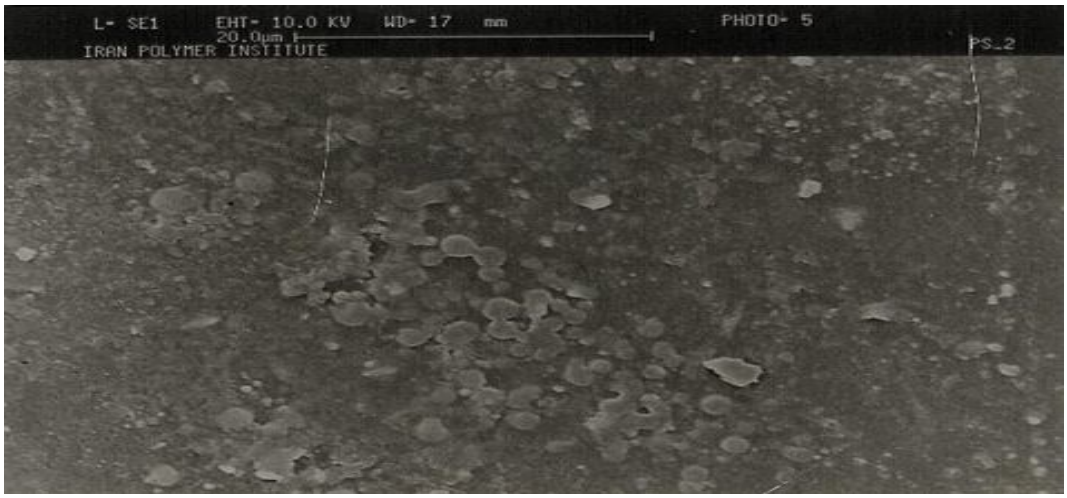


Figure 8. SEM Image of PS-*g*-PANi

The GPC of PS-*g*-PANi was analyzed using a polystyrene standard. The GPC curves obtained show a unimodal distribution. The molecular weight distribution averages for the polymer are presented in Table 1. High molecular weight of the polymer shows the growth of PANi on PS-*g*-PANi.

CONCLUSION

In our recent works, the grafted copolymers were synthesized from polypyrrole on polyvinyl acetate and polystyrene [8,9]. But polymers of solubility decreased by increasing the percentage of polypyrrole. We increased both conductivity and solubility by changing polypyrrole with polyaniline. As we described at the beginning of our discussion, grafting of aniline in polymers such as polystyrene and it is growth on chain, creates a conducting copolymer solution with a new physical properties. The produced PS-*g*-PANi have new physical properties, such as high flexibility, the ability to produce film, high conductivity, conductance of stability to air, sufficient thermal resistance and solubility in DMSO, DMF and m-cresol. The observed new properties of PS-*g*-PANi implies that this grafted copolymer is a better candidate for gas sensing.

REFERENCES

1. Hosseini, S.H., Entezami A.A., 1995. Synthesis of Conducting Polypyrrole and Polycarbazole Containing Hydroxamic Acid Groups, Euro Polym J., 32 (7):635-641.
2. Pron, A. Rannou, P., 2002. Processible conjugated polymers: from organic semiconductors to organic metals and superconductors, Prog. Polym. Sci., 27, 135-190.
3. Rubner, M.F., 1992. Molecular Electronics, Research Studies Press, chapter 2.
4. Alcacer L., 1987. Conducting Polymers, D. Reidel Publishing Company, chapter 1-4.
5. Naarmann H., 1993. Polymers to the Year 200 and Beyond, John Wiley & Sons, Chapter 4.
6. Ian, M., 1994. Introduction to Synthetic Polymers, Oxford Science Publications, Chapter 10.
7. Salaneck, W.R., Clark, D.T., Samuelsen, E.J., 1991. Science and Application of Conducting Polymers, IOP Publishing.
8. Hosseini, S.H., Entezami, A.A., 2003. Chemical and Electrochemical Synthesis of Conducting Graft Copolymer of Vinyl Acetate with Pyrrole and Studies of Its Gas and Vapor Sensing, J. Appl. Polym. Sci., 90, 40-48.
9. Hosseini, S.H., Ghavami, A., 2005. Graft Copolymers of Polystyrene and Polypyrrole and Studies of Its Gas and Vapor Sensing, Iranian Polym. J., 14(2), 101-110.
10. Hosseini, S.H., Dabiri, M., Ashrafi, M, 2006. Chemical and Electrochemical Synthesis of Conducting Graft Copolymer of Acrylonitrile with Aniline, Polymer International 55, 9: 1081-1089.
11. Kondo, S., Ohtsuka, T., Ogura, K., Tsuda, K., 1979. Convenient Synthesis and Free-Radical Copolymerization of p-Chloromethylstyrene, J. Macromol. Sci. Chem., A13(6), 767-775.
12. Pharhad Hussein, A.M., Kumar, A., 2003. Electrochemical synthesis and characterization of chloride doped polyaniline, Bull. Mater. Sci., 26 (3) 329–334.
13. MacDiarmid, A.G., Jones, W.E., Norris, I.D., Gao, J., and Okuzai, H., 2001. Electrostatically-generation nanofibers of electronic polymers, Synthetic Metals, 119, 27-30.
14. Bartlett, P.N., and Simon, E., 2000. Poly(aniline)-poly(acrylate) composite films as modified electrodes for the oxidation of NADH, Phys. Chem. Chem. Phys., 2, 2599-2606.
15. Hosseini, S.H., Entezami, A.A., 2005. Studies of Thermal and Electrical Conductivity Behaviors of Polyaniline and Polypyrrole Blends, Iranian Polym.J., 14 (3) 201-209.