Volume 8, Number 1, (Cumulative No.24) Part 3 February 15, 2011 ISSN:1097-8135

Life Science Journal

Marsland Press

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Website:
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Phone: (347) 321-7172

Life Science Journal 2011 Volume 8, Number 1, Part 3 ISSN:1097-8135

Volume 8, Number 1, Part 3 Februar y 15, 2011 ISSN:1097-8135

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MARSLAND PRESS
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ISSN: 1097-8135

Life Science Journal

Acta Zhengzhou University Oversea Version

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CONTENTS

Pyrethroid Toxic Effects on some Hormonal Profile and Biochemical Markers among Workers in Pyrethroid Insecticides Company

Sahar A. Abou El-Magd[1].; Laila M.E. Sabik.[2]*; and Amira Shoukry[3]

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Abstract: Background: As Pyrethroids use is common and likely increasing worldwide, so more researches are needed to know its hazardous effects.

Objectives: This study was designed to evaluate chronic toxic effects of synthetic pyrethroids on some hormonal profile (testosterone, estrogen, progesterone & thyroid hormones), respiratory system, liver and kidney functions, in addition, trying to clarify some underlying mechanisms of toxicity through measuring total antioxidant capacity, lipid peroxidation markers (malondialdhyde), and IgE among workers exposed to pyrethroids.

Subjects and Methods: The study included eighteen workers of both sexes exposed to pyrethroids in pyrethroid Insecticides Company. Twenty non exposed workers from the administrative workers of Faculty of Medicine Zagazig University were selected as a control group. All participating workers were interviewed using a pre-composed questionnaire, furthermore they were examined clinically and investigated by measuring some blood parameters as testosterone, estrogen, progesterone, thyroid hormones (T_3, T_4 and TSH), IgE, ALT, AST, creatinine, urea, total-antioxidants and malondialdehyde according to standard procedures.

Results: The studied groups were matched as regard gender, age, duration of work, marital status, income, residence and smoking habit. There was a highly significant prevalence of headache, cough & wheeze among exposed workers compared to control group ($p< 0.001$). Moreover, the exposed group had significantly lower values of testosterone, T_3, T_4, and pan-antioxidants, as compared to control group ($p<0.001$). Also, there was a higher significant values of TSH, IgE, ALT, AST and malondialdehyde among exposed workers as compared to control group ($p<0.001$).

Conclusion & Recommendations: Chronic exposure to pyrethroid insecticides may cause endocrine disrupting effects, respiratory problems, liver function impairment, beside oxidative stress and lipid peroxidation. So we recommended, improving working condition. Restriction of unlimited use of pyrethroid insecticides especially at home and agricultural purposes. Further researches are needed to evaluate pyrethroids effect on large sample to obtain detailed information about the exposure route, pathways, other mechanisms of toxicity and other health hazards.

[Sahar A. Abou El-Magd; Laila M. E. Sabik and Amira Shoukry. **Pyrethroid Toxic Effects on some Hormonal Profile and Biochemical Markers among Workers in Pyrethroid Insecticides Company**. Life Science Journal. 2011; 8(1):311-322] (ISSN: 1097–8135). http://www.lifesciencesite.com.

Keywords: Pyrethroids exposure, endocrine disruptor, lung, liver, kidney, oxidative stress.

1. Introduction

Pesticides are chemical substances that are used for the destruction of environmental organisms which are detrimental to people (Page, 1998).
Pesticide poisoning is an important cause of morbidity and mortality in developing countries. Every year there are 3 million cases of severe poisoning and 220,000 deaths; the majority of these poisonings and 99% of the resulting deaths occur in the third world (Tinoco and Halperin, 1998).

Pyrethroid pesticides are synthetic analogues of pyrethrins, which are natural chemicals found in chrysanthemum flowers. Although synthetic pyrethroids are based on the chemical structure and biological activity of the pyrethrins, the development of synthetic pyrethroids has involved extensive chemical modifications that make these compounds more toxic and less degradable in the environment (U.S. EPA, 2006*a*.&b).

While the use of pyrethroid insecticides has been documented since 1970s, preliminary evidence suggests that usage has been increasing and the pyrethroid insecticides are replacing the organophosphorus insecticides for residential control

(Jose, et al 2010). So the number of human exposures to organophosphorus insecticides decreased, while exposures to pyretheroid insecticides increased (Sudakine, 2006).

Diet is a primary route of exposure to pyrethroids among non-occupationally exposed individuals, particularly food containing pyrethroid residues e.g, vegetables and fruits (ATSDR, 2003). A high proportion of household dust samples contain pyrethroid residues, suggesting that the home environment may also comprise a major exposure source (Colt et al., 2004). Thus, exposure to pyrethroid insecticides is likely to be multi-media and multi-route, as occupational exposure to pesticides occurs also in the manufacturing process during preparation, transport, and application of these products. Exposure occurs among mixers, loaders, and applicators working in fields, greenhouses, parks, and among farm workers (Hernandez-Valero et al. , 2001). As the exposure to synthetic pyrethroids are extensive, animals exhibited changes in their physiological activities beside other pathological features, so the toxicity of pyrethroid insecticides to mammalian animals has received much attention in recent years (Sakr, 2003). Reproductive toxicity, endocrine disruption, neurodevelopmental toxicity and adverse immune system effects related to pyrethroids exposure have been reported in numerous studies (Wang et al., 2009).

Oxidative stress is a harmful process that can mediate damage to cell structures, including lipids, proteins, RNA and DNA which leads to a number of diseases (Saikat, 2010). Environmental agents, such as pesticides, initiate free radical generation that causes different complications in the body (Langseth, 1996).

Several biological defence mechanisms against intracellular oxidative stress are presented in the organism such as antioxidant enzymes (superoxide dismutase, catalase, glutathione reductase and glutathione transferase) and non-enzymatic antioxidants such as carotenoids, vitamin E, vitamin C and glutathione, can also act to overcome the oxidative stress of the pesticides (Evants and Halliwell, 2001).

This study was planned to evaluate chronic toxic effects of synthetic pyrethroids on some hormonal profile { testosterone, estrogen, progesterone & thyroid hormones, triiodothyronine (T_3) and thyroxine (T_4) and thyroid stimulating hormone (TSH) }, respiratory system, liver and kidney functions in addition, trying to clarify some underlying mechanisms of toxicity through measuring total-antioxidant capacity, lipid peroxidation markers (malondialdhyde), and IgE .

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2. Subjects and Methods:

Study design and setting

This comparative cross-sectional study was conducted from October 2009 to January 2010 at family company that makes leading global household pyrethroid insecticides products like:

* Baygon, its active ingredients, (Imiprothrine + Cyfluthrine) which is used as mosquitoes and cockroaches, multi-insect killer. This product is for export purpose.

*Raid and Baygon, their active ingredients (Imiprothrine + Deltamethrine) which are used as cockroaches and ant killer.

*Raid, its active ingredients (D-allethrine + Tetramethrine), which is used as flying insect killer.

* The company makes also other products like (Pledge, Mr Muscle, Shout, Glead and Windox). This company is at Al-Khanka district, Egypt.

Industrial process and exposure:

At preparation section a 500 kilograms of kerosene is withdrawn and heated till 45°C and then the active ingredient Tetramethrin is added and well mixed till complete solubility. Serdox, which is a non active material had added to increase particle size to allow aerosol properties. At the aerosol line the rest of kerosene has been withdrawn to tanks and synergic material is added. Emulsifier as span 80 is added, and lastly the active ingredient D-allethrine is added with continuous mixing and vigorous pouring till complete homogenicity. Finally, examination and supervision of the end product to be ready for commercial use and availability of wide varieties of brands and products.

N.B: Active ingredient for creeping insects (Imiprothrine , Deltamethrine) and for flying insects (D-allethrine ,Tetramethrine and Cyfluthrine).

System of work at the company:

The total number of persons in this company is 53 workers [39 workers (22males & 17 females), 7 technicians , 6 supervisors and the manager] . They work for 8 hours daily, starting from 8 AM to 12 PM in two daily shifts for 5days per week.

Subjects

Eighteen exposed and twenty control workers were agreeing to participate in this study, they are apparently healthy.

Exposed group:

- Eighteen exposed workers from both sexes are included in this study. Six males in the preparation section and twelve (Six males & six females) at the aerosol line section, they were selected according to the following inclusion criteria:

1) No previous (before joining the job) occupational or second job exposure to any type of insecticides.

2) Regular and direct exposure to pyrethroid insecticides emissions for at least three years.

Fig.1: Preparation section

Fig.2: aerosol line section

Control group:
Twenty workers were selected as non exposed control group from the administrative workers of Faculty of Medicine Zagazig University were included in this study according to the following criteria:
1- No previous occupational exposure to pyrethroid insecticides emissions.
2- Matching to the exposed group regarding age, gender, residence, socioeconomic standard, marital status, smoking habit, and duration of work.

* Exclusion criteria for both of the studied groups were
1-Free from viral hepatitis or liver cirrhosis.
2- No history of thyroid disease.
3- No history of drug therapy.
4- Not exposed to ionizing radiation in the last six months.
5- No current infections or cancer (at the time of the study).

Methods
Questionnaire
At first, the study protocol was approved by the *Ethics Committee of Faculty of Medicine, Zagazig University*, then after obtaining permissions from the manager of the company and written informed consents from all the participants, they were asked to fill out a pre-composed questionnaire and interviewed. A personal, occupational and past histories were taken to determine whether they have any medical or endocrinal problems, duration of work (at least 3 years), use of protective measures were also assessed.

Symptoms:
Identification of symptoms of exposure to pyrethroids as headache, cough & wheeze, dyspnea and repeated viral infection were reported according to *Ray and Fry, (2006)*.

2-Clinical examination: General and local examinations of both studied groups were carried out to detect any abnormalities.

3-Laboratory investigations:
*Samples collection:
A sample of 10cc venous blood was withdrawn from each worker under complete aseptic conditions. Blood samples were collected in test tubes remained to clot, centrifuged for obtaining serum samples then, kept at –20^{o}C until they were used to determine the following:
1- Estimation of serum testosterone, estrogen and progesterone levels (ng/ml):

Testosterone, estrogen and progesterone were assayed in serum samples by the use of Roche Elecsys reagent kit (Roche Diagnostica USA) and Modular analyzer used for assay *(Monath et al., 1995& Lu et al., 1999)*.

2- Estimation of serum T3, T4, and TSH: They were measured at Elecsys auto analyzer by Chem - iluminescence method according to *Grughn et al., (1987).*

3-Estimation of total immunoglobulins E (IgE): It was measured using enzyme linked immunosorbent assay (ELISA). Kits supplied by Clinotch Diagnostics and Pharmaceuticals, inc. *(Kulczyski, 1981).*

4- Liver function tests:

Aspartate transaminase and alanine transaminase activities (AST and ALT) were measured using spectrophotometer at a wave length (546 nm) according to Bergmeyer et al., (1978).

5- Kidney function tests:

Creatinine and Urea levels were measured using spectrophotometer a wave length (520 and 578 nm) respectively, according to Patton and Crouch, (1977) & Henry et al., (1974) respectively.

6- Determination of total antioxidant capacity: Total antioxidant capacity, new analytical test that may provide more relevant biological information compared to that obtained by the measurement of individual components as it represents the cumulative effects of all antioxidants either enzymatic or non-enzymatic, present in plasma and body fluids. It was measured using the Spectrophotometer at a wave length (500-510 nm) according to Koracevic and Koracevic (2001).

7- Determination of serum malondialdehyde (MDA): It was measured using the Spectrophotometer at a wave length (535nm) according to *Yoshioka et al., (1997).*

Statistical analysis:

The data were analyzed using the Statistical Package for Social Sciences (SPSS) version 10 software. Quantitative data were compared using student's t test and qualitative data were compared using chi-square (X^2) test or Fisher exact tests. Correlation test used to measure the relationship between quantitative variable. Results were considered significant when p-value < 0.05 (Norusis, 1997).

3. Results:

General and occupational characteristics:

The results of this study showed that there were no statistically significant differences between the studied exposed and the control groups as regard gender, age, duration of work, marital status, income, residence and smoking habit ($p>0.05$). It was found that majority of exposed workers participating in this study were at the aerosol line (66.67%), and half of them were using protective measures (table1).

* Prevalence of symptoms among the studied groups (table 2):

This study reveals that there was a highly significant prevalence of headache, dry irritative cough and wheeze among exposed workers compared to control group ($p<0.001$). Dyspnea and repeated viral infections showed no significant difference between the studied groups ($p>0.05$).

Blood parameters among the studied group:

Table (3) demonstrates that the exposed group had significantly lower values of testosterone, T_3, T_4, and total-antioxidants compared to the control group ($p<0.001$). Moreover, there was a higher statistically significant values of TSH, ALT, AST, IgE, and malondialdehyde (MDH) among exposed group compared to control group. ($p<0.001$).

It was found also that there were no significant difference between the studied groups as regard estrogen, progesterone, creatinine and urea ($p>0.05$). On comparing blood parameters of the exposed workers in preparation section to those in aerosol line section, it was found that AST was significantly higher in workers of preparation section than those of aerosol section (table 4).

Correlation between all studied parameters and duration of work among exposed group:

This study showed no correlation between all studied parameters and duration of work except for total-antioxidants which showed significant negative correlation with duration of work, which means decrement in total antioxidant with the increase in duration of work, Table (4).

Table (1): Comparison of general and occupational characteristics among the studied groups by Chi Squared and Fisher Exact tests.

Characteristics	Exposed workers n = 18	Control group n = 20	P
Gender			
• Male (N. %)	12 (66.67 %)	11 (55%)	0.46
•Female (N %)	6 (33.33 %)	9 (45%)	
Age (year) (X± SD)	36.29 ± 10.9	37.30 ±7.9	0.72
Duration of work (year) (X± SD)	13.38 ± 901	11.80 ± 6.7	0.50
Marital			
• Married	12 (66.67%)	12 (60%)	0.67
• Not Married	6 (33.33%)	8 (40%)	
*Income			
• Sufficient	16 (88.89%)	18 (90%)	1.00
• Not Sufficient	2 (11.11%)	2 (10%)	
Residence			
• Rural	11 (61.11%)	11 (55%)	0.7
• Urban	7 (38.89%)	9 (45%)	
*Smoking habit			
• Yes	2 (11.11%)	2 (10%)	1.00
• No	16 (88.89%)	18 (90%)	
Use of protective measures			
• Yes	9 (50%)		
• No	9 (50%)		
Work section			
• Preparation	6 (33.33%)		
• aerosol line	12 (66.67%)		

P: Non Significant * : Fisher Exact

Table (2): Comparison of prevalence of symptoms among the studied groups by Fisher Exact test.

Symptoms	Exposed workers	Control group	P
Headache			
• Yes	10 (55.56 %)	2 (10%)	
• No	8 (44.44 %)	18 (90%)	0.002*
Cough & Wheeze			
• Yes	6 (33.33 %)	1 (5%)	
• No	12 (66.67 %)	19 (95%)	0.0002*
Dyspnea			
• Yes	4 (22.22 %)	2 (10 %)	
• No	14 (77.78 %)	18 (90 %)	1.06
Repeated viral infections			
• Yes	3 (16.67 %)	1 (5 %)	
• No	15 (83.33 %)	9 (95 %)	0.24

***: p < o.oo1 highly significant**

Table (3): Comparison of blood parameters measurements in the studied groups by (t)test .

Blood Parameters	Exposed workers n = 18	Control group n=20	P
Testosterone (ng/ml) (♂ only)	16.97 ± 4.83 (n=12)	24.91 ± 3.72 (n=11)	0. 001*
Estrogen (Pg/ml) (♀ only)	105 ± 42.50 (n=6)	111 ± 59.44 (n=9)	0.48
Progesterone (Pg/ml) (♀ only)	2.17 ± 1.08 (n=6)	1.71 ± 0.47 (n=9)	0.11
T3 (mmol/L)	1.6 ± 0.21	2.77 ± 0.53	0.001*
T4 (mmol/L)	66.39 ± 9.2	84.25 ± 13.71	0.001*
TSH (ulU/ml)	3.38 ± 0.72	2.42 ± 1.35	0.001*
ALT (U/L)	23.61 ± 7.20	8.15 ± 1.95	0.001*
AST (U/L)	19.61 ± 6.47	12.15 ± 3.06	0.001*
Creatinine (mg/dl)	0.92 ± 0.24	0.75 ± 0.34	0.079
Urea (mg/dl)	30.05 ± 7.32	26 ± 4.95	0.091
IgE ((IU/ml))	158.35 ± 11.33	70.35 ± 20.45	0.0001*
Total- antioxidants (mU/L)	0.35 ± 0.24	1.37 ± 0.45	0.001*
Malondialdehyde (umol/ml)	28.20 ± 22.83	3.49 ± 1.01	0.001*

*** : p < o.oo1 highly significant**

Table (4): Comparison of blood parameters measurements in preparation section and aerosol line section by (t)test .

Blood Parameters	preparation section n = 6	aerosol section n=12	P
Testosterone (ng/ml) (♂ only)	15.98 ± 3.52 (n=6)	18.16 ± 6.29 (n=6)	0.48
T3 (mmol/L)	1.57 ± 0.15	1.62 ± 0.23	0.64
T4 (mmol/L)	64.66 ± 9.3	67.25 ± 9.42	0.58
TSH (ulU/ml)	4.23 ± 0.73	3.70 ± 0.68	0.15
ALT (U/L)	27.0 ± 4.77	21.91 ± 7.77	0.16
AST (U/L)	24.16 ± 3.86	17.33 ± 6.40	0.03*
Creatinine (mg/dl)	0.85 ± 0.32	0.96 ± 0.20	0.36
Urea (mg/dl)	29.16 ± 6.49	30.50 ± 7.93	0.72
IgE ((IU/ml))	152.70 ± 11.39	161.17 ± 10.64	0.13
Total- antioxidants (mU/L)	0.43 ± 0.19	0.31 ± 0.26	0.36
Malondialdehyde (umol/ml)	39.46 ± 31.21	22.57 ± 16.10	0.14

***: p < o.05 significant**

Table (5): Correlation between total- antioxidants (mU/L)and duration of work (year) in the exposed group.

	Total- antioxidants (mU/L)	
	r	P
Duration of work (year)	-0.49	0.036*

***: p < o.05 significant**

4. Discussion:

Insecticides are the chemicals widely used in agriculture, environmental health, human-and animal-health fields. Exposure to insecticides has been associated with many hazardous effects *(Kanbur et al., 2008).* The widespread use of pyrethroids and the corresponding increase in human exposure have led to toxicological interest *(Kolaczinski and Curtis, 2004).* Several studies have proven that pyrethroids are endocrine disrupting insecticides (EDs). An "endocrine disrupting chemical" is best defined as "an exogenous substance that causes adverse health effects in an intact organism, or its progeny, secondary to changes in endocrine function *(EEC, 1996).* Many of the endocrine disrupting pesticides are active *in vivo* at extremely low doses which can be made by the permitted residue levels in food *(Weltje et al., 2005)* or exposure to low levels of EDs, the effects of which can be additive *(Soto et al., 1994).* Testosterone levels and sperm counts in men have reportedly declined during the last 20 years *(Travison* et al*., 2007).* Low testosterone levels have been shown to contribute to low bone and muscle mass, impaired sexual function, and decreased fertility (Thomas et al., 2008).

Environmental chemicals are suspected of playing a role in these declines *(Swan* et al*., 2003).* Synthetic pyrethroid insecticides are among the most commonly used chemicals today *(John et al., 2009).*

The results of the present study demonstrated that serum testosterone levels is significantly lower in pyrethroid exposed workers compared to the control group, these findings are in accordance with *Zhang et al., (2007)* who stated that the widely-used synthetic insecticide Permethrin dramatically reduces testosterone levels and sperm counts in adult male mice. Another study in non-occupationally exposed men, reported statistically significant relationships between pyrethroid insecticide metabolite concentrations and circulating testosterone hormone levels. They attributed these findings to the increased use of pyrethroid pesticides that results in widespread exposure among the general population (*John et al., 2009).*

The results of the present study could be explained by *Melissa et al., (2007)* Who reported that pyrethroids as a class of non-steroidal compounds, can interact competitively with human androgen receptors and sex hormone binding globulin, and suggest a mechanism by which chronic exposure to pyrethroid may result in disturbances in endocrine effects relating to androgen action, as it may exert estrogenic and/or anti-androgenic activity. The same findings were reported before by *Eil and Nisula, (1990)* when pyretheroid compounds (Pyrethrins and Bioalletherine) were tested in human genital skin fibroblasts.

Zhang et al., (2007) found that Permethrin causes reproductive damage by altering the beginning steps of testosterone synthesis in the mice testes that leading to lowering testosterone production in the testes and blood.

In the current study, there was no significant difference between exposed and control groups as regard serum estrogen and progesterone, as Pyrethroids-induced estrogen disrupting effects didn't interfere with serum estrogen hormone levels. For example, Cypermethrin, Deltamethrin and their metabolites (3-phenoxybenzoic alcohol & 3-phenoxy benzoic acid) exhibited significant estrogenic activities comparable to 17B-estradiol (E_2) when they were evaluated for their estrogenic activities in the MCF-7 human breast carcinoma cell line *(Jin et al., 2010).*

Synthetic pyrethroids referred to as xenoestrogens which are a diverse group of substances that do not necessarily share any structural resemblance to the natural hormone 17B-estradiol (E_2). However, they may exert oestrogenic effects by mimicking or inhibiting the action of endogenous estrogens by their ability of binding to the estrogen receptors, and therefore inducing or attenuating a response *(Kojima et al., 2004).*

In this work, the pyrethroid exposed workers had a significantly lower triiodothyronine (T_3) and thyroxine (T_4) serum levels as well as a significantly higher thyroid stimulating hormone (TSH) serum levels when compared to control group.

Our results are in a accordance with *Akhtar et al., (1996), Maiti and Kar, (1998), Wang et al., (2002) and Finch et al., (2006)* who found decreased serum levels of both T_3 and T_4 and increased serum levels of TSH in experimental rats exposed to different synthetic pyretheroid compounds.

Maiti and Kar, (1998), has explained that there is also decrease in the activity of hepatic type I iodothyronin 5´-monodeiodinase (5´ D-I) which is one of the deiodinase enzymes that convert T_4 to the more potent T_3 with consequent decrease in T_3 and elevation in TSH serum levels.

Moreover, *Finch et al., (2006) have* otherwise attributed the increase of TSH levels to pyrethroid induced increase in hepatic microsomal thyroxine UDP glucuronosyl transferase activity which leads to increased glucuronidation and elimination of thyroxine. Consequently, a compensatory increase in pituitary gland production of TSH, and also increase in thyroid gland production of thyroid hormones should occur to keep up with the elimination.

Finch et al., (2006) added that this is the same mechanism underlying pyrethrins – induced rat thyroid gland tumors as the trophic effects of TSH occurs in the form of increased thyroid gland weight, follicular cell hypertrophy and replicative DNA synthesis. Thus, Pyrethrin-induced thyroid gland tumors are similar to that of some other non-genotoxic inducers of hepatic xenobiotic metabolism.

The results of the present study revealed that the exposed workers complain of some respiratory symptoms like cough, wheeze, shortness of breath and dyspnea. These symptoms coincide with increase in IgE level, when compared with the control group.

The results of the present study pass in parallel with *He et al., (1998),* who stated that there is some evidence that pyrethroid compounds are sensitizers in human populations.

Clinical studies involving insecticide-sensitive patients with asthma have suggested that some asthmatics have declines in lung function due to exposure to insecticide aerosols containing Permethrins (Salome et al. , 2000).

Other occupational and agricultural studies have reported positive associations between Permethrin pesticide exposure and wheeze or asthma in adults *(Hoppin et al., 2006 & 2008).*

Reardon et al., (2009) reported that higher pre-natal levels of *Cis*-permethrin were associated with early cough, wheeze, and IgE production. .

Martinez et al., (1995), explained that early wheeze can be transient and attributed to viral infections, whereas persistent wheeze is more likely to have an underlying allergic component.

In this study there was a significant increase in the serum levels of aspartate transaminase (AST) and alanine transaminase (ALT) in the pyrethroid exposed workers as compared to control group.

These findings coincide with Al-sarar et al., (2009) who reported a slight elevation in AST, ALT and ALP serum levels in pesticides-exposed workers of Riyadh municipality, KSA. Significant increase in the levels of these enzymes, which is also positively correlated with pesticide residues, were found in occupationally exposed tobacco farmers in Pakistan *(Khan et al., 2008).* The increase in the level of ALT and/or AST is a good indicator of hepatic toxicity *(Hall, 2001).*

Recent experimental studies have shown that Lambda-Cyhalothrin increases the enzymatic activities of aminotransferases AST and ALT, which is ameliorated with co-administration of vitamin C *(Fetoui et al., 2010).* Cypermethrin, a synthetic pyrethroid insecticide, have been shown to increase liver enzymes and produce necrosis of hepatocytes cytoplasmic vaculation, bile duct hyperplasia and mononuclear cellular infiltration in the liver of broiler chicks which is ameliorated by combination of Vitamin E and selenium *(Aslam et al., 2010).*

The results of this work revealed normal kidney function (urea & creatinine) in exposed worker.

These findings coincide with *Al-Sarar et al., (2009)* who found insignificant elevation in urea and creatinine among pesticide sprayer in Riyadh ,who exposed to both pyretheroid and organophosphorus , and with *Satpathy et al. ,(1997)* who found no toxic effects on renal function among adult males after short-term exposure to Cyfluthrin.

In contrast to our findings, two laboratory studies showed that male kidneys of mice and rats may be particularly susceptible to synthetic pyrethroid (Sumithrin) (Cox, 2003). In our opinion the conflict with our findings may be due to route of exposure as animals in those studies were fed Sumithrin for two generations.

Normal kidney functions reported in our study means that kidney functions is still good and compensated, our opinion explained before by Feinfeld, (1998) who found that at least 50% of kidney function must be lost before the rise of serum creatinine could be detected.

The results of the present work revealed a significant decrease in total antioxidant capacity of exposed workers in addition, a significant increases in malondialdehyde (MDH) level, compared to the control group.

The results of the present study pass parallel with *Vontas et al., (2001), Cinzia et al., (2004), Sadowska et al., (2010)* ,Who stated that pyrethroid exposure associated with oxidative stress, as it induced lipid peroxidation , protein oxidation and depleted multiple antioxidant enzymes like, reduced glutathione , glutathione peroxidase, catalase and superoxide dismutase activities.

Kanbur et al., (2008) found that, the degree of oxidative stress and lipid peroxidation induced by pyrethroid, related to the dose administered, the duration of exposure and the administration of the indicated compounds, either alone or as a combination.

A predominance of reactive oxygen species (ROS) production and DNA damage can contribute to cytotoxicity of Cis-bifenthrin (synthetic pyretheroid insecticide), *Wang, et al., (2009).* The depletion in total antioxidant capacity as well as the increment in MDH (lipid peroxidation marker) could be explained by *Banerjee et al., (2001)* who suggested that the formation of oxygen free radical can be a major factor in the toxicity of pesticides. On the other hand, *Nasuti et al (2003) and Prsanthi et al., (2005)* reported that oxidative damage, induced by pyrethroids might be

due to their lipophilieity, whereby they could penetrate easily to the cell membrane and caused membrane lipid peroxidation.

5. Conclusion:

Despite of being the least toxic pesticides, pyrethroids still have a harmful effects, as chronic exposure to pyrethroids can cause endocrine disrupting effects, liver function impairment and respiratory problems. Oxidative stress, lipid peroxidation and allergy may be some underlying mechanisms of toxicity.

Although workers in preparation section are considered more exposed to pyrethroids than those in aerosol line section, there was no significant differences between them in the studied hormonal and biochemical parameters except for AST enzyme. This may be attributed to wearing of the protective clothes (specialized overalls, gloves and shoes) specially during the preparation process, however they neglect wearing masks.

Recommendations:

As there are worldwide exposure to Pyrethroids which may be environmental, occupational or at home so we recommended the following :

Improving working conditions and following hygienic measures, beside supplementation of antioxidants to workers to overcome oxidative stress.

Restriction of unlimited use of pyrethroid insecticides especially at home or for agricultural purposes .

Periodic examination of Pyrethroids exposed workers both clinically and laboratory for early detection of any abnormalities.

Further researches are needed to evaluate pyrethroids effect on large samples to obtain detailed information about the exposure route, pathways, metabolites, other mechanisms of toxicity, and other health hazards.

Acknowledgment

We should thank all workers for their cooperation and understanding. And special thanks to the manager of this company for his help to complete this study. Special appreciation to Dr. Amal Fathy, assistant professor of biochemistry, Faculty of Medicine, Zagazig University, for her help in the laboratory investigations of this study.

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12/26/2010

Deterioration and Treatment Study of Archaeological Limestone Statues, Sakkara, Egypt

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Abstract: Sakkara is one section of the great necropolis of Memphis, the Old Kingdom capital and the kings of the 1st Dynasty as well as that of the 2nd Dynasty are mostly buried in this section of the Memphis necropolis. A group of Greco-roman limestone statues were found in front of Serapeum tomb entrance which was built at least as early as the 18th dynasty. They called "Statues of Philosophies". They suffer from different deterioration phenomena such as missing parts, erosion of stone, presence of cracks and micro cracks, disintegration of some parts, crystallization of salts and dirt. Deterioration factors were different sources of moisture, salts, wind erosion and changes in temperature. Studying phenomena and factors of their deterioration were performed by various investigations and analyses. Stone samples were collected from these statues. study of these samples have been carried out using polarizing microscope (PLM), scanning electron microscope (SEM), X-ray diffraction (XRD) and energy dispersive X-ray analysis (EDX). Treatment and conservation techniques were discussed. XRD data showed that limestone consists mainly of calcite. (PLM) examination showed presence of fine-grained calcite in stone texture. (SEM) determined disintegration between mineral grains, erosion, loose of binding material, cracks and salts crystallization. Restoration techniques of the Greco-roman statues were studied, for example mechanical and chemical cleaning , consolidation, treatment of cracks , compilation missing parts and isolation of the surface to protect it against moisture using water repellent materials.
[S. A. Abd El – Rahim and M.K. Khallaf. **Deterioration and Treatment Study of Archaeological Limestone Statues, Sakkara, Egypt**. Life Science Journal. 2011;8(1):323-328] (ISSN:1097–8135). http://www.lifesciencesite.com.

Key Words: Sakkara; serapeum; Statues of Philosophies; limestone consolidation; water repellent materials.

1- Introduction:

Serapeum is a name usually applied to building that was associated with the cult of the Apis bulls, or the later composite God, Serapis. (Taylor, 2004). We actually know of two Serapeums, one located at Saqqara and the other in Alexandria. (Malek, 1983). The one at Saqqara was more closely related to the Apris bulls, while the Alexandria Serapeum served as a cult center of Serapis. In reality, these two complexes served very different purposes, the Serapeum in Alexandria being more Greek in origin, while the one at Saqqara was built at least as early as the 18th dynasty. This study focuses on the earlier structure at Saqqara. (Mathieson, et al., 1997). Napoleon's expedition had searched for the Serapeum in vain, but archeologist Mariette discovered the complex in 1850, in the early days of archeology. (Farag, 1975). Mariette was led to the site of the Serapeum through his discovery of traces of some of the sphinxes (over 100) lining the dorms, that were faithfully described by the Greek writer Strabo. (Dodson, 2000). In Le Serapeum de Memphis. Excavation carried out in 1852 revealed an older gallery known as the "lesser Vaults". They had similar rock hewn chambers that had contained bulls in wooden coffins. (Mathieson, et al., 1999). They dated from year 30 of Ramesses II reign down to the 22nd Dynasty. (Taylor, 2004). The burial of Apis XIV made in the 44th year of Ramesses II reign survived intact. Throughout 1952, Mariette's work continued resulting in the discovery of a thrid series of smaller bull burials. (Dodson, 2000). They ranged in date from Amenophis III of the 18th Dynasty through the 19th dynasty, the earliest burials found. Here, two coffins that of Apis VII and Apis IX was also discovered intact, along with shabtis, canopic jars and amulets. One of the Apis bulls can be found in the Cairo Agricultural Museum. (Taylor, 2004). "Statues of Philosophies" suffer from different deterioration phenomena such as missing parts, erosion of stone surface, many cracks, disintegration of some parts, crystallization of salts and accumulated dirt (Fig. 1). Deterioration factors were different sources especially daily and seasonally changes in humidity and temperature degrees and wind erosion. (Fronteau, et. al, 2010).

2- Filed observation

The statues suffer from different deterioration phenomena such as missing parts, erosion of stone surfaces, different type of cracks; macro, micro and wide deep cracks, disintegration of many parts, crystallization of salts and dirt. Deterioration factors were different sources of moisture, salts, wind erosion and changes in temperature and moisture degrees. On the other hand

they suffer from high degradation as the following: high failure, fragile and flaking off due to high moisture and crystallization of salts (Del Monte and Sabbioni, 1986) in addition to a bad state of their conservation (Fig. 2).

Fig (1): The place of Statues of Philosophies and its preservation status, Sakkara, Egypt

Fig. (2) Details of deterioration phenomena of the statues as surfaces degradation, crystallization of soluble salts, dark layers on the surfaces, vertical and horizontal deep cracks, erosion by wind effect and missing parts.

3- Materials and Methods

Stone samples were taken from the statues. The investigations of the studied samples were performed using the following methods:

3.1. Optical microscopy

Samples were first observed using optical light microscopy (Zies, Japan) and then the thin sections were examined using polarized transmitted light microscopy (Olympus BX41) attached with digital camera under 40 x magnifications in plane-polarised and crossed-polarised light.

3.2. Scanning electron microscopy

Scanning electron microscopy (SEM) investigations of the samples were carried out by Philips (XL30) microscope, equipped with EDX micro-analytical system to obtain the total element content in the samples.

3.3. X-ray diffraction

Fine powders of the samples were analysed with a diffractometer (Philips, PW 3710, CoKα 40 kV, 30 mA).

4- Results and Discussion

4.1 Polarizing Microscope (PLM)

Examination of thin sections of the limestone samples under plan polarised light microscope (PLM) displayed a fine-grained calcite crystals besides presence of iron oxides, quartz, clay minerals and fossils (Fig. 3). These components increase the rate of stone decay.

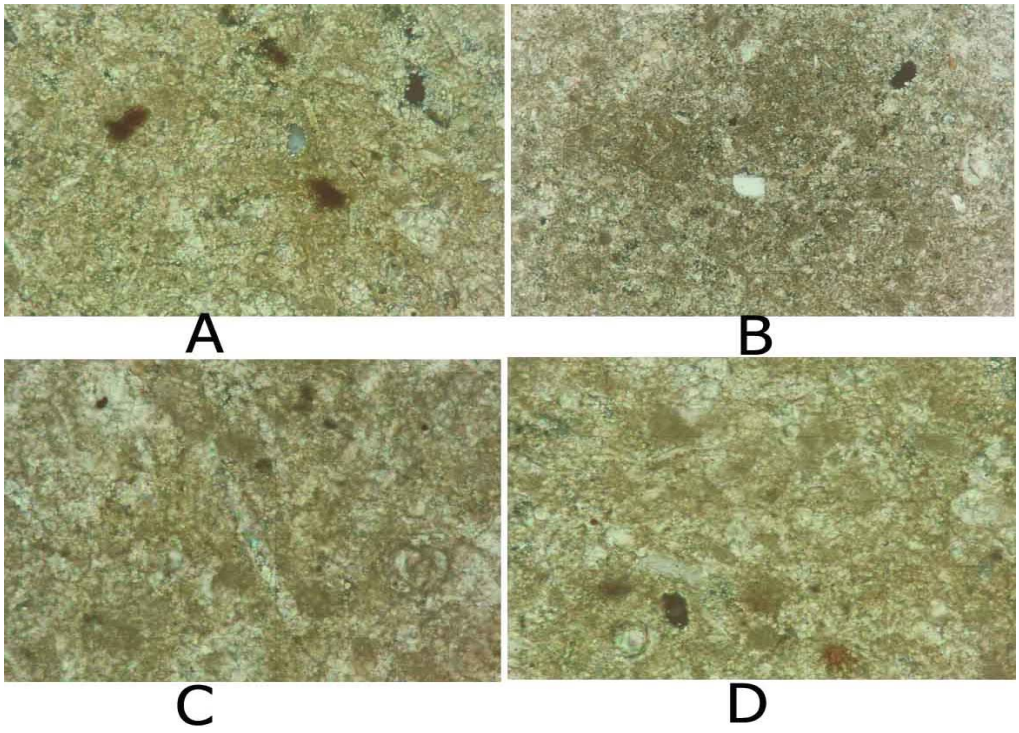

Fig (3. A-D): Shows fine grained calcite (micrite), iron oxides, clay minerals and some fine grained quartz.

4.2 Scanning Electron Microscope (SEM) equipped with Energy dispersive X-ray analysis (EDX).

SEM photomicrographs showed disintegration between calcite crystals. Lose in the binding materials between grains by the effect of salts crystallization and erosion by wind effect (Fig 4A-D)

EDX analysis was taken of the fresh unpolished cross-section. The cross-section showed the presence of calcite composed mainly of (Ca), (C), and (O). Small amounts of silicon (Si) due to quartz mineral, iron (Fe) related to iron oxides, aluminium (Al) as clay minerals, sodium (Na) and chlorine (Cl) due to presence of halite salt were found (Fig. 5). The moisture can migrate into and within a limestone sculptures in variety ways, depending on whether it is in the liquid or vapor state (Fitzner, et al., 1997) and the transformation of moisture involving variety processes, such as absorption, evaporation, diffusion and capillarity as well as the surface tension of the liquid (Inta, 1996). Furthermore presence of soluble salts within a porous material (Angeli, et al**.**, 2010) such as limestone will increase the amount of water and there are two main mechanisms that responsible for the introduction of soluble salts into the building including of the Statues: capillary rise of groundwater and infiltration by rainwater result in different deterioration phenomena. (Laho, et al., 2010).

4.3 X-Ray Diffraction analysis (XRD)

The XRD analysis of the yellow limestone sample consists of calcite ($CaCO_3$) as a major component in addition to dolomite ($Ca,Mg(CO_3)_2$), and halite (NaCl) as traces (Fig. 6)**.** Salts can absorb moisture, especially when the relative humidity increases above their equilibrium RH. (Beck, 2010) Then, particularly the very soluble ones may deliquesce (i.e., absorb so much water vapour) that they form a saturated solution. (Charola, 1988) The term "deliquescent" is preferable to "hygroscopic" to describe this phenomenon because it identifies the condition of becoming liquid (Aneta, and Richard, 2010). The actual distribution of moisture within stone depends on porosity, pore-size distribution, and environmental conditions such as changes in temperature and effect of wind with sand. (Garland and Rogers, 1995) As discuss, the maximum of moisture content resulting from wet-dry cycling is closer to the surface in denser stones, deeper and

broader in coarse porous materials. (Beck and Mukhtar. 2010).

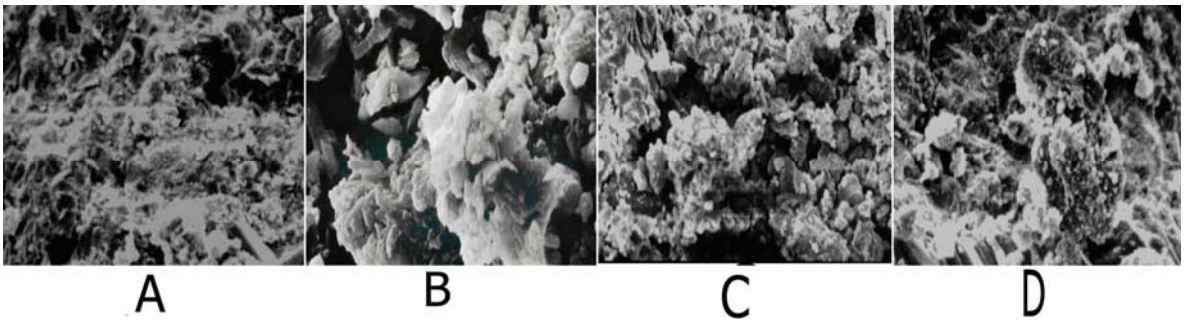

Fig (4A-D): SEM photomicrographs showing the collapse of internal structure (A), voids (B), loose of binding material (C) and salts crystallization between mineral grains of limestone(D).

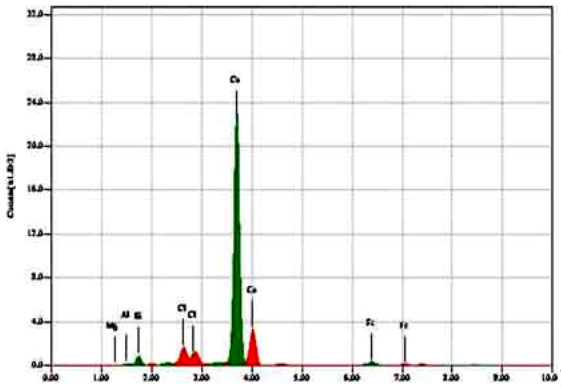

Fig. (5): EDX pattern of limestone sample

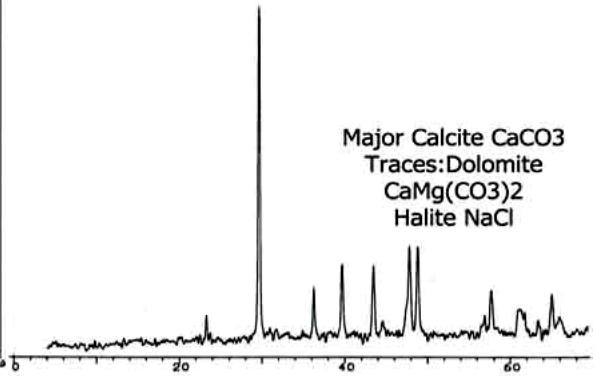

Fig. (6): XRD pattern of limestone sample

6- Treatment and conservation suggestions

The statues need to carry out different treatments and conservation processes which include cleaning of statues surfaces, removal and extraction of salts, stone in-fill, consolidation and water repellent treatments. These processes will be carried out as follows:

6.1 Cleaning Processes

The reasons for cleaning of the statues are to improve the appearance of them by removing unattractive dirt or soiling materials. (Bede, 2000). Mechanical cleaning firstly will carry out using manual and mechanical tools. Water cleaning methods are generally the gentlest means possible, and they can be used safely to remove dirt from the surfaces of the statues supplemented with non-ionic detergent; and steam, or hot-pressurized water cleaning. (Mack and Grimmer 2003). Chemical cleaners react with dirt, soiling material or paint to effect their removal, after which the cleaning effluent is rinsed off the masonry surface with water (Angeli, et al., 2010). Non-ionic detergents (which are not the same as soaps) are synthetic organic compounds that are especially effective in removing oily soil. (Laho, et al., 2010). The addition of a non-ionic detergent, or surfactant, to a low- or medium-pressure water wash can be a useful aid in the cleaning process. Steam cleaning is actually low-pressure hot water washing because the steam condenses almost immediately upon leaving the hose. This is a gentle and effective method for cleaning stone and particularly for acid-sensitive stones. (Ana, et al., 2010). It can also be an efficient means of cleaning carved stone details and, because it does not generate a lot of liquid water. (Angeli, et al., 2010) These are usually of much the same composition as other alkaline cleaners, containing potassium or ammonium hydroxide, or tri-sodium phosphate. (Laho, et al., 2010). They are used to remove oil, latex and acrylic paints, and are effective for removing multiple layers of paint. The formulation of organic solvent paint removers varies and may include a combination of solvents, including methylene chloride, methanol, acetone, xylene and toluene. (Beck and Mukhtar2010)

6.2 Removal and Extraction of Salts

The most common poultice medium is clay, although paper and cotton fibers are also used, and talc, chalk and even flour are traditional poultice materials. (Laho, et al., 2010). A mixture of clay and paper fiber produces an absorbent and plastic mixture that is often favored by conservators of stone sculpture. (Aneta and Richard, 2010).This plain or true poultice is normally used for desalination, to draw out soluble salts, or as a cleaning method on substrates such as limestone that respond to water cleaning. (Angeli, et al., 2010) In these cases the poultice is allowed to dry out and the soiling and/or salts are drawn into the poultice by capillary action with the moisture (Ana, et al., 2010). Multiple applications may be necessary to draw the salts from within the surface pores. Whatever the medium, the poultice is mixed with water to form a material that will adhere to the substrate (Angeli, et al., 2010) Clay forms a sticky mass that adheres well to stone and other surfaces. These plain poultices can be conveniently mixed by hand as required on site with the addition of water to the poultice medium. (Danuta, et al., 2010).

6.3 Stone in fill

The mortar of stone infill for the statues missing parts and cracks treatment consists of hydraulic lime, limestone powder and sand grains (250 um), 1: 0.25: 3 parts by wt. in order. The stone should be well matched to the original fabric in terms of color texture and finish. (Domaslowski, and Strzelczyk1993). The infill will be secured into place with stainless steel reinforcements (in large areas) and laid wet with a bedding mortar. (Gansicke and Hirx, 1997). The mortar should packed into place on a clean humid surface the mortar will be applied in successive layers not exceeding 2 cm in thickness which must cure before the new layer is applied for deep cavities stainless steel reinforcement will be used the repair will be finished with a damp sponge brush after curing . (Laho, et al., 2010).

6.4 Consolidation Process

Stone strengtheners based on ethyl silicates are generally applied by spraying or flooding (Clifton, 2005). Several wet – on – wet treatments are generally needed applied at intervals of 20 to 30 minutes, (Beck and Mukhtar, 2010). The exact number of treatments quantity of material and desired minimum penetration depth have to be ascertained by preliminary tests and trials. (Ana et al., 2010). The construction materials must be dry since the active in gradient in the stone strengthener, i.e., the ethyl silicates reacts with moisture. The moisture required by the stone strengthener for chemical deposition of the silica gel is supplied by the construction material which always has a certain sorption moisture content varying in equilibrium with the atmospheric humidity. (Noll, 1986). The best working conditions are a relative humidity of 40 to 70 % and a surface temperature on the construction material of 10 to 25^{O} c each coating operation be so arranged that the entire surface can be covered in one working day. (Aneta and Richard2010).

6.5 Water-Repellent Coatings

Water-repellent coatings are formulated to be vapor permeable, or "breathable". (Laho, et al., 2010). They do not seal the surface completely to water vapor so it can enter the masonry wall as well as leave the wall. Now most water-repellent coatings are water-based and formulated from modified siloxanes, silanes and other alkoxysilanes. (Wheeler, 2005). While some of these products are shipped from the factory ready to use, other water-borne water repellents must be diluted at the job site. (Mack, and Grimmer 2003). Unlike earlier water-repellent coatings which tended to form a "film" on the stone surface, modern water-repellent coatings actually penetrate into the stone substrate slightly and, generally, are almost invisible if properly applied to the statues. They are also more vapor permeable than the old coatings, yet they still reduce the vapor permeability of the stone. (Ana et al., 2010).

7- Conclusion

Deterioration, cracks, erosion and disintegration between mineral grains of the stone Statues occur by wind with sand erosion, moisture, changes in temperature and soluble salts migration. The investigations and analyses of samples showed that the crystalline chlorides exert a pressure on the pores which cause disintegration and cracking of stone and change it into a brittle mass in some parts. Many parts of the stone were missed. The stone contains clay minerals and iron oxides which increased deterioration of the Statues.

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12/21/2010

Laboratory Approach To Chlamydia Trachomatis Conjunctivitis

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Abstract: It is a chronic follicular conjunctivitis. In Egypt, the high prevalence of trachoma represents a major cause of blindness especially in rural areas. The aim of this work is to confirm the clinical diagnosis and to evaluate the enzyme linked immunosorbent assay in direct antigen detection of C.trachomatis in conjunctival scrapings and C.trachomatis antibodies in the sera of patients. Two groups of patients; the first group included 20 active cases (group I) and the second group included 25 cicatricial cases (group II). Direct antigen detection by ELISA from conjunctival scrapping, trachoma IgG and IgM by ELISA. Evaluation of direct antigen detection of C.trachomatis in conjunctival scrapings by ELISA revealed that there were insignificant difference between active and cicatricial ($P>0.05$). There was insignificant higher titre in active than cicatricial cases. As regard, IgM detection of C.trachomatis there were insignificant difference between them ($P>0.05$). There was insignificant higher titre in active than cicatricial cases. Detection of C.trachoatis IgG revealed 20 positive cases (44.4%), all of them were cicatricial cases (80%) which were significantly higher than active cases ($P<0.001$). There was significantly higher titre in cicatricial than in active cases. All antigen positive cases in group I were bilaterally affected, while in group II, detection of C.trachomatis antigen was higher in unilateral than bilateral eye infection. There was insignificant difference between active and cicatricial cases in either affection ($P>0.05$) with insignificant higher titre in bilateral than unilateral positive cases. The sensitivity of ELISA IgM compared to direct antigen detection in cicatricial cases was 50%, the specificity was 100%. Direct antigen detection test and serodiagnosis of C.trachomatis IgM by ELISA are more reliable than ELISA IgG in diagnosis of active trachoma infection. ELISA IgG is a reliable method in the serodiagnosis of cicatricial phase of trachoma.

[Elham Ragab Abd El Samea, Eman Badr El Din El Hadidy, Sahar Mostafa El Tarshouby, Amina Mostafa Abd El Aal: Laboratory study of Chlamydia trachomatis conjunctivitis. Life Science Journal. 2011;8(1):329-3336] (ISSN:1097–8135). http://www.lifesciencesite.com.

Keywords: Chlamydia Trachomatis, Conjunctivitis, Trachoma

Introduction

Six million people-most of whom live in crowded, unhygienic conditions in developing countries- are blind because of an infectious disease called trachoma. It is generally accepted that trachoma is caused by Chlamydia trachomatis, bacteria that pass easily between people on hands and clothing[(1)]. Infection usually occurs first during childhood, but people do not become blind until adulthood. Successive infections cause progressive scarring of the inside of the eyelid. Eventually, the eyelashes turn inward and rub painfully over the front of the cornea. This causes corneal scarring, loss of corneal transparency and, finally, irreversible blindness. However, C.trachomatis and other organisms appear to be developing drug resistance to antibiotics commonly used to treat these infections. In addition, early scarring and in-turned eyelashes can be treated surgically. The World Health Organization has been promoting these "SAFE" interventions (surgery, antibiotics, facial cleanliness, and environmental improvement) since 2001 with the aim of eliminating trachoma by 2020. However, these control measures have had limited success so far and it looks like a vaccine may also be needed. To develop an effective vaccine, scientists need to know whether all cases of human trachoma are caused by so-called ocular strains of C.trachomatis. Might C.trachomatis strains that are usually associated with sexually transmitted disease or different species in the family Chlamydiaceae also cause human trachoma[(2)]**.** It is chronic follicular conjunctivitis caused by infection with Chlamydia trachomatis serovars A, B, Ba and C**.** Trachoma remains a serious health problem despite of great advances in therapeutic regimen. In Egypt, the high prevalence of trachoma represents a major cause of ocular morbidity and blindness especially in rural areas. The disease is often neglected because of illiteracy and real diagnostic difficulty thus delaying therapeutic intervention. Pannus is a common ocular intrachoma and punctuate keratitis[(3)]**.** Variable methods for diagnostic of trachoma had been widely discussed in recent years. Until a decade ago, the complement fixation test measuring group specific antibody was the most widely applied technique. However, despite

showing high sensitivity in diagnosis of systemic chlamydial infections, it had a little value in diagnosis of localized infections such as trachoma or inclusion conjunctivitis[4]. The aim of this work is to diagnose Chlamydia trachomatis as an important cause of trachoma to confirm the clinical diagnosis and to evaluate the enzyme linked immunosorbent assay in direct detection of C.trachomatis antigen in conjunctival scrapings and C.trachomatis antibodies in sera of trachomatous patients.

Subject and Methods

The study was carried out on 45 cases having clinical signs of trachoma and included two groups of patients; the first group included 20 active cases ranging from 4 – 12 years (group 1) and the second group included 25 cicatricial cases ranging from 36 – 72 years. They were out patients in Mansoura ophthalmic center after establishment of clinical diagnosis. *Specimen collection for C. trachomatis antigen detection:* Vigorous scraping from the upper tarsal conjunctiva were taken and placed in the transport medium which is sucrose phosphate saline. Antibiotics were added aseptically; amphotricin B, Gentamycin and vancomycin. Specimens were vortexed or sonicated to help disrupting cell depris and releasing chalmydial elementary antibodies, and the swabs were removed under complete aseptic conditions. Then the specimens were stored at -70°C to preserve the viability of organism. *For C. trachomatis antibody detection (IgM & IgG):*Serological specimens were collected aseptically; 2ml blood were taken from each patient and were put in dry test tube and after separation of the serum, serum samples were stored at -20°C. *Screening test for diagnosis of Chlamydia trachomatis conjunctivitis:* Direct antigen detection in conjunctival scrapings by ELISA using Mastazyme-Chlamydia test method*;* Detection of Chlamydia trachomatis IgM and IgG in serum by ELISA using Genozyme Virotech GmbH test method.

Results

Table (1) shows detection of C. trachomatis antigen, IgM and IgG by ELISA in studied 45 cases. Antigen detection of C. trachomatis was positive in 7 cases; 3 active cases and 4 cicatricial cases with insignificant difference between them (P>0.05).IgM detection of C. trachomatis was positive in 5 cases; 3 active cases and 2 cicatricial cases with significant difference between them (P>0.05). While detection of C.trachomatis IgG revealed 20 positive cases, all of them were cicatricial cases which were significantly higher than active cases (P<0.001).

Table (2) shows positive ELISA titre of C.trachomatis antigen, IgM & IgG. C.trachomatis antigen detection showed insignificant higher titre in active than cicatricial cases (P>0.05). Also, C.trachomatis IgM showed insignificant higher titre in active than cicatricial cases (P>0.05). In contrary, ELISA IgG titre was significantly higher in cicatricial than active (P<0.001).

Table (3) shows comparison between ELISA IgM and direct antigen detection in active cases. The sensitivity of ELISA IgM compared to direct antigen detection was 66.7%, the specificity was 94.1%. The negative predictive value was higher (94.1%) than the positive predictive value (66.7%). The accuracy was 90%. The percentage of false positive and false negative results was 33.3% and 5.9% respectively.

Table (4) shows comparison between ELISA IgM and direct antigen detection in cicatricial cases. The Sensitivity of ELISA IgM compared to direct antigen detection was 50%, the specificity was 100%. The positive predictive value was higher (100%) than the negative predicitive value (91.3%). The accuracy was 92%. The percentage of false positive and false negative results was zero% and 8.7% respectively.

Table (5) shows comparison between ELISA IgG and direct antigen detection in active cases. The sensitivity of ELISA IgG compared to direct antigen detection was zero, the specificity was 100%. The positive and negative predictive values were zero, 85% respectively. The accuracy was 85%. The percentage of false positive and false negative results was 100% and 15% respectively.

Table (6) shows comparison between ELISA IgG and direct antigen detection in cicatricial cases. The sensitivity of ELISA IgG compared to direct antigen detection was 75%, the specificity was 19.1%. the negative predictive value was higher (80%) than positive predictive value (14.3%). The accuracy was 28%. The percentage of false positive and false negative results was 85.7% and 20% respectively.

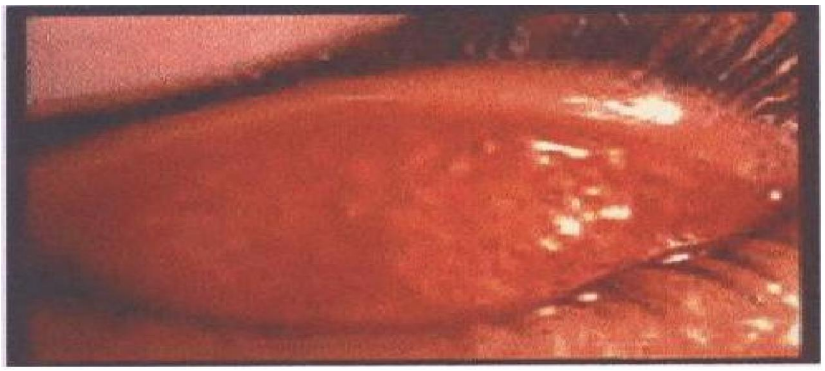

Photo (1): Active phase of trachoma (Grade I)

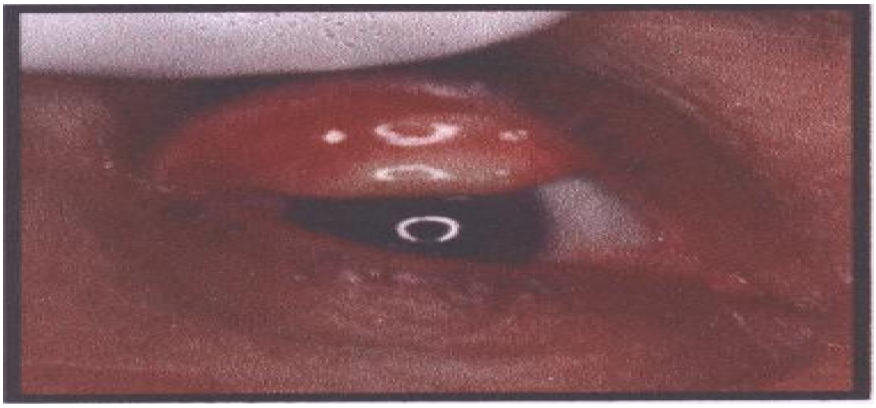

Photo (2): Cicatricial phase of trachoma (Grade III)

Table (1): Detection of C. trachomatis antigen, IgM & IgG by ELISA in studied cases (45).

Test	Total (n=45)				Active cases Group I (n=20)				Cicatricial cases Group II (n=25)				P
	Positive		Negative		Positive		Negative		Positive		Negative		
	No	%	No	%	No	%	No	%	No	%	No	%	
ELISA test: Antigen detection	7	15.6	38	84.4	3	15	17	85	4	16	21	84	>0.05
IgM	5	11.1	40	88.9	3	15	17	85	2	8	23	92	>0.05
IgG	20	44.4	25	55.6	0	0	20	100	20	80	5	20	<0.001

Table (2): Positive ELISA titre of C.trachomatis antigen detection, IgM & IgG.

Test	Active Cases Group I (n=20)				Cicatricial cases Group II (n=25)				P
	NO	%	Mean±SD	Range	NO	%	Mean±SD	Range	

ELISA test: Antigen detection	3	15	0.091±0.058	0- 0.22	4	16	0.062±0.182	0.01-0.68	>0.05
IgM	3	15	0.177±0.093	0.01-0.37	2	8	0.140±0.063	0.01-0.3	>0.05
IgG	0	0	0.036±0.029	0.01-0.13	20	80	0.192±0.147	0.01-0.43	<0.001

Table (3): Comparison between ELISA IgM and antigen detection in active cases

	ELISA antigen Detection				Sensitivity	Specificity	Accuracy	P.P Value	False +ve	N.P Value	False -ve
	positive		negative								
	No	%	No	%							
ELISA IgM:											
+ve	2	66.7	1	5.9	66.7%	94.1%	90%	66.7%	33.3 %	94.1 %	5.9%
-ve	1	33.3	16	94.1							

Table (4): Comparison between ELISA IgM and antigen detectin in cicatricial cases

	ELISA antigen Detection				Sensitivity	Specificity	Accuracy	P.P Value	False +ve	N.P Value	False -ve
	positive		negative								
	no	%	No	%							
ELISA IgM:											
+ve	2	50	0	0	50%	100%	92%	100%	zero	91.3%	8.7%
-ve	2	50	21	100							

Table (5): Comparison between ELISA IgG and antigen detection in active cases

	ELISA antigen Detection				Sensitivity	Specificity	Accuracy	P.P Value	False +ve	N.P Value	False -ve
	positive		negative								
	No	%	No	%							
ELISA IgM:											
+ve	0	0	0	0	Zero	100%	85%	Zero	100%	85%	15%
-ve	3	100	17	100							

Table (6): Comparison between ELISA IgG and antigen detection in cicatricial cases

	ELISA antigen Detection				Sensitivity	Specificity	Accuracy	P.P Value	False +ve	N.P Value	False -ve
	positive		negative								
	no	%	No	%							
ELISA IgM:											
+ve	3	75	17	80.9	75%	19.1%	28%	14.3 %	85.7%	80%	20%
-ve	1	25	4	19.1							

Discussion

Chlamydia trachomatis is a unique obligate intracellular bacterium that is the leading cause of bacterial sexually transmitted and blinding disease worldwide. Trachoma is a chronic disease of the conjunctival mucosa that can lead to blindness 10-40 years after infection. The estimated number of people with trachoma who will develop blindness by the year 2020 is 12 million[(2)]. Postoperative rates of trichiasis recurrence are high even with treatment for C.trachomatis at the time of surgery[(5)]**,** and C.trachomatis infection rates return within a year or two following cessation of mass or targeted antibiotic treatment programs. The latter may in part be due to an accelerated rate of reinfection following azithromycin treatment, which may blunt the immune response to the organism and lead to a population with increased susceptibility to infection[(6)]**.** Trachoma is one of the earliest recorded disease, it is a chronic follicular conjunctivitis caused by Chlamydia trachomatis serovars A, B, Ba and C[(7)]**.** Trachoma remains serious health problem despite of great advances in therapeutic regimen. The disease affects about 500 million people worldwide and is considered to be among the most important human chronic infections and most common cause of preventable blindness today[(8)]. In Egypt, the high prevalence of trachoma represents a major cause of ocular morbidity and blindness especially in rural areas. The disease is often neglected because of illiteracy and real diagnostic difficulty thus delaying therapeutic intervention[(9)]. The Nile Delta of Egypt represents a unique environment for trachoma to persist. Although economic improvements in last decade have affected even the poorest rural environments, the poor hygienic conditions still the primary factor in trachoma transmission**.** Enzyme immunoassays had been evaluated as rapid screening tests for diagnosing Chlamydia trachomatis conjunctivitis. They had the potential advantages of simplicity and objectivity; they are also easy, inexpensive and allow for large scale screening in endemic populations. Moreover, they do not depend on the presence of viable Chlamydiae during handling, transportation or storage of specimens[(10)]**.** This study was carried out on 45 cases having clinical signs of trachoma and including two groups of patients; the first group included 20 active cases, and the second group included 25 cicatricial cases. All cases were subjected to conjunctival scrapings for direct antigen detection of C.trachomatis by ELISA test, serum sample were also taken to detect antichlamydia trachomatis antibodies (IgM & IgG) by ELISA. The prevalence of trachoma was totally higher in female patients than in male patients. In cicatricial cases, trachoma was found in 16 cases female patients compared to 9 cases male patients. This was in agreement with ***Mabey et al.,***[(11)] who found a high prevalence of active trachoma in young female children. They also stated that trichiasis and blindness due to cicatricial trachoma may be 2-4 times more common in adult women than in men due to prolonged contact of women with children in active infection during child bearing age. Active trachoma was found in 65% of rural setting. ***Shehmann et al.,***[(12)] noticed a high relationship between spread of trachoma among children in rural area of Burkina Faso with the presence of flies and poor community hygiene. They observed that flies were present on 80% of children faces who had active infection. ***Broman AT, et al.,***[(13)] detected a low prevalence of active trachoma (15.6%) from total of 178 cases in rural Tanzania. ***Lansingh et al.,***[(14)] found that grade I; trachomatous follicles was higher (79%) than grade II; trachomatous inflammation (37%) on studying a trachoma survey in school children less than 10 years of age. Another study conducted by ***Madani et al.,***[(15)] revealed lower prevalence of active trachoma in the same age group; 31.5% trachomatous follicles (grades I) and 16.7% trachomatous inflammation (grade II). ***Katz et al.,***[(16)] studied the prevalence and severity of trachoma in school children and found that cicatricial trachoma was not present among this age group. However, ***Lansingh et al.,***[(14)] detected trachomatous scarring in 23% of

children in the same age group. Evaluation of direct antigen detection of C.trachomatis in conjunctival scrapings by ELISA revealed 7 positive cases; 3 active cases and 4 cicatricial cases with insignificant difference between them (P>0.05). There was significant higher titre in active than cicatricial cases, similar results were reported by ***Mabey et al.,***[17]. Also, ***Adenis et al.,***[18] found that out of 73% trachoma cases, 19.2% had a positive ELISA results for C.trachomatis antigen. A national survey was conducted by ***Saal et al.,***[19] to determine the prevalence of trachoma in children under the age of 10 years and the estimated prevalence of active infection by ELISA was 10.8%. ***Zhang et al., 1995***[20] studied 63 patients with severe active trachoma using enzyme linked immunosorbent assay for chlamydial antigenicity and detected a positive result in 97% of studied cases. This high prevalence of C.trachomatis antigen could be attributed to the severity of the disease. The appearance of antichlamydial antibodies in sera and tears of patients with trachoma after experimental eye infections is well documented, specific antibodies of IgM, IgG and IgA classes were detected[21]**.** Following an initial infection with C.trachomatis, the serum antibody response is usually of the IgM type that appears in two weeks and persists for a period of four to eight weeks. The IgG antibody usually appears late and persists for longer periods [22]**.** IgM detection of C.trachomatis was positive in 5 cases (11.1%); 3 active cases (15%) and 2 cicatricial cases (8%) with insignificant difference (P>0.05). There was insignificant higher titre in active than cicatricial cases. Our results were in harmony with ***Garg et al.,***[23] who detected antichlamydial IgM antibodies in 17% of patients suffering from active trachoma eye infection. Also, ***Abdel Rahman et al.,***[24] found serum IgM antibodies in 16.6% of patients with active infection. These data were also consistent with ***Numazaki et al.,***[25] who detected serum IgM in 13.2% of Japanese children with active inflammatory trachoma. The presence of IgM antibodies by ELISA may facilitate the diagnosis of an early infection and is particular helpful in infants. Reinfection with homologous trachoma serovar results only in anamnestic response in IgG antibodies without stimulating the IgM type, whereas reinfection with a new serovar results in an IgM antibody response to the new type as well as an anamnestic IgG rise to the previous one. Since most ocular infections in endemic areas are caused by the closely related serovars, it is not surprising that IgM antibody could be found only in a small percentage of patients from which the organism was isolated in cell culture[26]. Tear IgG usually exeeds the IgA titres and both are lower than serum titres in the same patient. Also IgG antibody was detectable in conjunctival secretions only when it was also present in serum, suggesting the possibility of transudation from the serum to conjunctival secretions through inflamed conjunctiva[27]. In the current study, detection of C.trachomatis IgG revealed cicatricial cases (80%) which were significantly higher than active cases (P<0.001). ELISA IgG titre was significantly higher in cicatricial than active cases. Our results were parallel with ***Hermann et al.,***[28] who found high prevalence of IgG antibodies (88%) in adult sera with cicatricial trachoma. Moreover, ***Numazaki et al.,***[25] reported that by means of ELISA, 71.3% of cicatricial cases aged more than 50 years had significantly elevated levels of IgG antibodies. ***Hessel et al.,***[29] stated that antichlamydial IgG antibodies were reactive in 90% of patients with follicular trachoma and 89% with inflammatory trachoma on examination of tear samples from Nepali villagers. The sensitivity of ELISA IgM in active cases was 66.7%, the specificity was 94.1%. The negative predictive value was higher (94.1%) than the positive predictive value (66.7%), the accuracy was 90%, ***Haller et al.,***[30] reported similar ELISA IgM sensitivity (70%) and lower specificity (78.9%). Also, ***Numazaki et al.,***[25] found a higher ELISA IgM sensitivity (81%) and similar specificity (92%). The sensitivity of ELISA IgM in cicatricial cases was 50%, the specificity was 100%. The positive predictive value was higher (100%) than the negative predictive value (91.3%). The accuracy was 92%, this result was previously reported by ***Pearce and Gaston,***[26] who stated that ELISA IgM had a sensitivity of 41.7% in diagnosis of cicatricial trachoma. They concluded that the role of ELISA IgM can be restricted to the detection of early cases and usually not detected in chronic infections. The sensitivity of ELISA IgG in active cases was zero, the specificity was 100%. The positive and negative predictive values were zero and 85% respectively. The accuracy was 85% . ELISA IgG in cicatricial cases had a sensitivity of 75% and a specificity of 19.1%. The negative predictive value was higher (80%) than the positive predictive value (14.3%), the accuracy was 28%. ***Haller et al.,***[30] found that ELISA IgG had a higher sensitivity (92%) and specificity (100%), the positive predictive value was 96% and the negative predictive value was 94%. ***Schachter et al.,***[31] stated that ELISA IgG had a higher sensitivity (90%) and specificity (98.9%). The high specificity in the previous studies may be attributed the difference in the antigen utilized which was the major outer membrane protein of C.trachomatis compared to LPS used in this study. Reevaluation of treatment regimens and approaches to vaccine development may be required as over II genomes of the order chlamydials have been sequenced[32]**,** infection with multiple species as C. psittaci and C.pneumoniae in addition to C.trachomatis in trachoma may explain also failure to detect Chlamydia among active trachoma

cases when only C.trachomatis is assayed ***Dean et al.,***[(2)]

It could be concluded that, application of ELISA IgM method in the serodiagnosis of active trachoma infection while IgG method is recommended during the cicatricial phase of the disease. In bilaterally affected patients it is better to obtain bilateral scrapings to provide more chance for antigen detection. Detection of secretory IgA in tear would be helpful in measuring the prevalence and intensity of active trachoma infection. Further evaluation of other techniques for C.trachomatis antigen detection such as culture method and polymerase chain reaction is recommended.

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30/11/2010

Zinc and Boron Fertilization on Concentration and Uptake of Copper and Nitrogen in Corn Grain in a Calcareous Soil

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Abstract: A farm experiment was conducted to study the effect of Zn and B interaction on the concentration and total uptake of Nitrogen (N) and Copper (Cu) in corn grain at Fars Province, Iran. Treatments including five levels of Zn (0, 8, 16 and 24 kg ha^{-1} and Zn foliar spray) and four levels of B (0, 3, and 6 kg ha^{-1} and B foliar spray) in a completely randomized block design were set up. A high Zn content in the soil helped increasing the concentration and uptake of N in the grain by B application; that is, at high levels of Zn, there was a synergism between B and N. Boron spraying helped with increasing the concentration and uptake of N in the grain by Zn application. There was a negative correlation between N and Cu concentration in the grain and a positive correlation between N and Cu uptake in the grain.
[F. Aref. Zinc and boron fertilization on concentration and uptake of copper and nitrogen in the corn grain in a calcareous soil. Life Science Journal. 2011;8(1):337-343] (ISSN:1097–8135). http://www.lifesciencesite.com.

Keywords: Antagonism, Deficiency, Fertilizer, Interaction, Nutrients, Synergism

1. Introduction

Nitrogen is present in a part of any protein compounds, any enzyme, metabolism intermediate compounds, compounds involved in production and transfer of matter and energy and even in the structure of ribonucleic acid that conducts the transfer of genetic traits. In addition to being involved in the structure of proteins, it makes up a part of the chlorophyll as well. Nitrogen is the first nutrient the deficiency of which being referred to when speaking about soils in arid and semi-arid regions. Sufficient supply of N is associated with high vegetative growth and dark green color (Tisdale et al., 1993). The N content in plant organs is next to carbon, oxygen and hydrogen (Tisdale et al., 1993). Agafone (1991) has reported that by increasing the use of N fertilizers, the plant's need to Cu will increase. Nitrogen causes an increase in uptake and concentration of Cu in the wheat the reason of which is attributed to a change in soil pH and, consequently, an increase in the solubility of Cu in the soil solution, increase in root volume and its extension, as well as the synthesis of compounds that may be carriers for Cu uptake. The plant's Cu is mostly involved in enzymatic activities. Its presence is necessary in enzymatic oxidase – catalase systems. Also, this element is involved in electron transfer reactions and is the activator of several enzymes (Tisdale et al., 1993). It is also involved in the metabolism of proteins and carbohydrates and N fixation (Pals and Benton, 1997). A correct balance between Zn and Cu concentrations plays a significant role in their uptake level. There are many reports on zinc-copper interaction in references. Copper is necessary for protein production. Therefore, adding it to soils with Cu deficiency leads into an increase in their protein content. Pals and Benton (1997) state that the Cu uptake level is lower than most micronutrients.

Based on a report by Dhillon et al. (1987), in the Zn deficiency conditions, conversion of N to protein compounds is reduced and the buildup of amino acids and amids in the plant under these conditions is an evidence for the importance of Zn in protein synthesis. According to a report made by Price et al (1972), Zn deficiency in the plant is associated with RNA and ribosome reduction the result of which is a defect in protein synthesis and consequently, build up of free amino acids in the plant. Ribosomes are located on the cell RNA and are involved in protein production. Nuttal et al. (1987) report that the joint use of B and S increases the grain protein content while joint B and N use reduces the protein content and increases the oil content. Boron is effective in the metabolism of N compounds in the plant and in its deficiency, soluble N compounds, especially the nitrates, build up in the plant (Marschner, 1995). Increase in the Zn level, affects the N uptake and production in the plant (Dhillon et al. (1987). There are many reports on the effect of N on Zn uptake (Karimian, 1995; Mishra and Singh, 1996). Gupta and Patalia (1993) reported that Zn application had no effect on N uptake if N fertilizer was not used, while with using N, application of Zn increased N uptake. They attributed the increase in N uptake to an increase in the dry weight of the airborne organs. Due to the role of Zn in RNA and protein synthesis, Zn use increases the effectiveness of N in the plant (Kitagishi et al., 1987).

Hussien and Faiyad (1996) reported that by Zn application, the plant's N concentration increased. Gupta et al. (1986) observed that with the use of 2.5 mg Zn/kg soil, the N uptake by corn increased from

53.4 mg in the control to 206.2 mg in the fertilized treatment. Many authors have reported the effect of Zn on the increase in N uptake by the plant (Salam and Subramanian, 1988; Sahu et al., 1996). Latife (1983) reported that by using zinc sulfate, the plant height, ear length and diameter, the number of grains per ear, the weight of 1000 grains, grain yield and the N uptake significantly increased. Kumar et al. (1981) stated that adding Zn to the soil reduced Fe, Mn and Cu concentration and increased Zn concentration in the plant. Many reports are made by authors on zinc–copper interaction (Cayton et al. 1983). Some authors reported that Zn use increased plant growth and reduction of Cu concentration in corn and cereals. Some authors attribute the antagonism of Cu and Zn uptake to having common uptake sites on the root surface and some have attributed the same to their antagonism in transfer of one another from the root to airborne organs (Cayton et al. 1983; Mesquita, 2000). Parker et al. (1992) showed that Zn application increased Mn concentration in the plant but had no effect on Fe and Cu concentrations. Gupta (1993) has reported an interaction between N and B.

The grain being rich in nutrients, including N and Cu plays an important role in human nutrition. The grain being rich in such elements is also an evidence for improve of the harvest, qualitatively and quantitatively. Therefore, by studying Zn and B interaction in the grain, we can find its indirect effects that arte improvement of the harvest, qualitatively and quantitatively, while enriching the grain as well. Also, it has been shown that if we use grains rich in these elements as seeds, we can improve the harvest, qualitatively and quantitatively.

The objective of the study was to evaluate the concentration and uptake of N and Cu in corn grain as affected by Zinc sulfate and Boric acid application.

2. Materials and Methods

A field experiment was conducted at the farm of Firouzabad University, Fars province of Iran, on the corn (*Zea mays L.*), cultivar "Single Cross 401" during 2010 cropping season. Before implementing the project sampling from the soil (0-30 cm depths) was made in order to select a zone in which the available amount of Zn and B was low (less than 1mg kg^{-1} extracted by methods DTPA and hot water, respectively). This soil had a loam texture, pH of 8.4, 0.78 % organic matter, 210 mg kg^{-1} exchangeable potassium (K), 9.9 mg kg^{-1} available phosphorus, DTPA extractable Fe, Mn, Zn and Cu concentration were 1.4, 6, 0.38 and 1 mg kg^{-1} and available B with hot water extractable was 0.9 mg kg^{-1}.

Firouzabad located on latitude 28°51'17"N and longitude 52°31'58"E and 1330 m altitude. The soil at the experimental site was a loam. This experiment included 20 treatments and 3 replications in the form of completely randomized block design and factorial that combinations of five levels Zn (0, 8, 16 and 24 kg ha^{-1} Zn, and Zn solution spray) and four levels of B (0, 3, and 6 kg ha^{-1} and B, and B solution spray). Due to a high pH and the high calcium content of the soil in question, a high level of Zn was used. Nitrogen: P: K used at 160, 95 and 100 kg ha^{-1} according to the recommendation, from sources of urea (46% N), triple super phosphate (46% P_2O_5) and potassium sulfate (50% K_2O), respectively, were added to all treatments (plots). Moreover, 50% of the urea was used when planting and the remainder two times: At vegetative growth and when the corn ears were formed. Zinc and B, from zinc sulfate (35 percent Zn) and boric acid (17 percent B) sources, respectively, were used by two methods, adding to the soil and spraying. Addition to the soil was made at the time of plantation and the sprayings were made at 5 per thousand zinc sulfate and 3 per thousand boric acid two times: one at vegetative growth stage and the other after corn ears formation. The Zn and B were both applied to the leaves with uniform coverage at a volume solution of 2500 L/ha using a knapsack sprayer. Each experimental plot was 8m length and 3m width, had 5 beds and 4 rows, equally spaced, and seeds 20cm apart on the rows.

Analysis of the grain and soil was carried out using common lab procedures. Phosphorous in soil was measured by Olsen method, available K by acetate ammonium extraction method and potassium assessment in the extract by flame photometer, organic carbon by the Walkley and Black method. Available Fe, Zn, Mn and Cu in the soil were first extracted by DTPA and then were read by atomic absorption setup. The soil's available B was extracted by hot water and then was measured by spectrophotometer by curcamin method, considering the intensity of the color produced. For N determination, dried grain was digested with sulfuric acid and was analyzed by semimicro- Kjeltec methods (Bremner and Mulvaney, 1982). Digestion method by dry burning was used to measure Cu and then they were measured by atomic absorption setup. Statistical analysis of data was made using SAS software with Duncan test.

3. Results and Discussion

3.1. Soil analysis before culture

The results of soil analysis before culture are summarized in table 1. The P, K and Zn in the soil were below the critical level but B was at the medium to deficiency level. Manganese, Cu and Fe of the soil

were at higher than the critical level. Karimian and Ghanbari (1990) reported the critical P level by Olsen method in calcareous soils to be 18 mg kg^{-1}. Sims and Johnson (1991), reported the critical limits of soil's Fe, Zn, Mn and Cu by the DTPA extraction method and B by the hot water method to be 2.5-5. 0.2-2, 1-5, 0.1-2.5 and 0.1-2 mg kg^{-1}, respectively. Agrawala (1992) reported the critical level of Fe, Zn, Mn and Cu in the soil by the DTPA extraction to 2.5, 0.8, 5.5 and 0.75 mg kg^{-1} soil, respectively.

Table 1. Soil mechanical and chemical analysis

Properties	Values
Depth of soil(cm)	0 -30
Soil texture	Loam
pH	8.4
EC (ds m^{-1})	2
Organic matter (%)	0.78
Nutrients (mg kg^{-1})	
P	9.9
K	210
Fe	1.5
Mn	6
Zn	0.38
Cu	1
B	0.9

3.2. Nitrogen concentration in the grain

The effects of Zn and B on the grain N concentration were insignificant at a 5% level (table 2). The study of the effect of Zn and B interaction on the grain N concentration showed that B use only at 24 kg ha^{-1} Zn level increased the N concentration in the grain. Application of 3 kg ha^{-1} B at 24 kg ha^{-1} Zn increased the grain N concentration from 1.5 to 1.8 percent (20% increase as compared with no B use at this Zn level) while other B levels showed no significant effect. At other Zn levels, application of B had no effect on grain N concentration. Zinc spraying use only at the B solution spraying level increased grain N concentration from 1.55 to 1.99 percent, showing a 28.38 percent increase as compared with the no Zn use level. The lowest and the highest leaf N concentration, 1.5 and 1.99%, were observed at using 24 kg ha^{-1} Zn and joint Zn and B spraying levels, respectively. Except for these two treatments, other treatments showed no significant difference from the control.

3.3. Nitrogen uptake by the grain

The main effect of Zn on N uptake by the grain (kg ha^{-1}) was significant at 5% level (table 3). The lowest mean N uptake by the grain at 125.99 kg ha^{-1} was seen at no Zn level. With applying 16 and 24 kg ha^{-1} Zn, N uptake by the grain increased from 125.99 at zero Zn level to 155.92 and 148.57 kg ha^{-1}, respectively (23.76 and 17.92 percent increase, respectively), but no significant difference was seen between these two Zn levels. Zinc spraying increased grain N uptake to 155.79 kg ha^{-1}, showing a 23.65% increase relative to zero Zn level; but there was no significant difference between the Zn spraying and applying Zn to the soil in that regard. The highest N uptake by the grain, which is 155.92 kg ha^{-1}, was seen at 16 kg ha^{-1} Zn level.

The effect of applying different B levels on N uptake by the grain was significant at 5% level. The lowest grain N uptake, 129.07 kg ha^{-1}, was seen at zero B level. Boron use at all levels (applying to the soil and spraying) increased grain N uptake relative to zero B use. The use of 3 and 6 kg ha^{-1} B increased grain N uptake from 129.07 at zero B level to 150.78 and 148.56 kg ha^{-1}, respectively (16.82 and 15.1 % increase, in that order); but there was no significant difference between these two B levels in that regard. Boron spraying, too, increased grain N uptake from 129.07 to 155.26 kg ha^{-1}, a 20% increase relative to zero B level; but no significant difference was seen between Zn spraying and its addition to the soil in that regard. The highest grain N concentration was due to B spraying.

Studying the effect of Zn and B interaction on grain N uptake showed that B use at highest Zn level (24 kg ha^{-1} Zn), increased grain N uptake but at other Zn levels, it showed no significant effect on the N uptake. At 24 kg ha^{-1} Zn level, the use of 3 kg ha^{-1} and B spraying significantly increasing grain N uptake from 115.65 kg ha^{-1} to 165.99 and 174.53 kg ha^{-1}, respectively (43.52 and 50.91 percent increase relative to zero B use), but the use of 6 kg ha^{-1} B had no significant effect.

Zinc use at B spraying level, significantly increased grain N uptake but at other B levels, had no significant effect on N uptake. At B spraying level, application of 16 and 24 kg ha^{-1} Zn, increased grain N uptake from 116.43 to 171.9 and 174.53 kg ha^{-1}, respectively (47.64 and 49.9 percent increase, respectively). Zinc spraying at B spraying level, too, increased grain N uptake to 169.38 kg ha^{-1} (45.48% increase) but no significant difference was seen with the case in which Zn was directly applied to the soil.

The lowest N uptake by the grain, 108.83 kg ha^{-1}, was seen in the case where no Zn and no B was used (the control). The highest N uptake by the grain, 174.9 kg ha^{-1}, was seen when 6 kg ha^{-1} B + 16 kg ha^{-1} Zn was used, leading to 60.71% increase relative to the control.

Table 2. The effect of Zn and B on the N concentration (%) by the grain*

B (kg ha^{-1})	Zn (kg ha-1)					
	0	8	16	24	Foliar Spray	Mean
0	1.62 bcd	1.6 bcd	1.69 bcd	1.5 d	1.75 abcd	1.63 a
3	1.76 abcd	1.63 bcd	1.65 bcd	1.8 abc	1.67 bcd	1.72 a
6	1.83 ab	1.64 bcd	1.81 abc	1.69 bcd	1.71 bcd	1.74 a
Foliar Spray	1.55 cd	1.66 bcd	1.76 abcd	1.7 bcd	1.99 a	1.73 a
Mean	1.69 ab	1.63 b	1.73 ab	1.67 ab	1.78 a	

*Means with same letters lack a significant difference at 5% level by Duncan's test

Table 3. The effect of Zn and B on the N uptake (kg ha^{-1}) by the grain*

B (kg ha^{-1})	Zn (kg ha^{-1})					
	0	8	16	24	Foliar Spray	Mean
0	108.83 b	138.69 ab	128.51 ab	115.65 b	153.68 ab	129.07 b
3	145.2 ab	148.07 ab	148.37 ab	165.99 a	146.28 ab	150.78 a
6	133.5 ab	142.45 ab	174.9 a	138.09 ab	153.84 ab	148.56 a
Foliar Spray	116.43 b	144.08 ab	171.9 a	174.53 a	169.38 a	155.26 a
Mean	125.99 b	143.32 ab	155.92 a	148.57 a	155.79 a	

*Means with same letters lack a significant difference at 5% level by Duncan's test

3.4. Copper concentration in the grain

The main effect of Zn and B on the grain Cu concentration was not significant at a 5% level (table 4). In the study of the effect of Zn and B interaction on the grain N concentration, it was observed that at 8 kg ha^{-1} Zn, application of B only in spray form reduced grain Cu concentration from 4.67 to 2.67 mg kg^{-1} (42.82% reduction) but at other Zn levels, B use had no significant effect on the grain Cu concentration. Zinc application had no effect on Cu concentration in the grain at any B levels.

No treatment showed a significant difference from the control. The highest and the lowest grain Cu concentration, 2.67 and 4.67 mg/ha, showed 19.82% reduction and 40.42% increase as compared with 3.33 mg kg^{-1} of the control.

3.5. Copper uptake by the grain

The main effect of Zn and B on the grain Cu uptake was not significant at a 5% level but the effect of Zn and B interaction was significant at 1% level (table 5). Boron application in the form of spraying at 8 kg ha^{-1} Zn level reduced Cu uptake by the grain from 39.6 to 23.13 g/ha (41.59% reduction) but application of B directly to the soil had no significant effect. At a high Zn level (24 kg ha^{-1} Zn), only B spraying increased Cu uptake by the grain from 26.43 g/ha to 44.57 g/ha (68.63% increase) but at other Zn levels, B application showed no significant effect on uptake. Probably a high soil Zn content (24 kg ha^{-1} Zn) reduced B toxicity and, consequently, an increase in Cu uptake by the grain with B application; but a low Zn level (8 kg ha^{-1} Zn) was not able to reduce the toxicity and, consequently, the Cu uptake by the grain with B application was reduced.

Zinc use in cases where B was not applied directly to the soil (zero and B spraying levels) increased Cu uptake by the grain but in cases where it was applied directly to the soil (3 and 6 kg ha^{-1} B), it had no significant effect on Cu uptake by the grain. At zero B level, only application of 8 kg ha^{-1} Zn significantly increased Cu uptake by the grain from 22.53 to 39.6 g/ha (75.76% increase). The use of 24 kg ha^{-1} Zn at B spraying level increased Cu uptake by the grain from 25.17 to 44.57 g/ha (77.07% increase) but other Zn levels had no significant effect. Probably

due to a Zn and B antagonism, B application prevented from Zn use affecting Cu uptake by the grain.

Table 4. The effect of Zn and B on the Cu concentration (%) by the grain*

B (kg ha^{-1})	Zn (kg ha^{-1})					
	0	8	16	24	Foliar Spray	Mean
0	3.33 abcd	4.67 a	4 abc	3.33 ab	3 bc	3.67 a
3	4 abc	4.33 ab	4 abc	2.67 c	3.67 abc	3.73 a
6	3.67 abc	3.33 abc	2.67 c	3 bc	3.67 abc	3.27 a
Foliar Spray	3.33 abc	2.67 c	3.33 abc	4.33 ab	3 c	3.33 a
Mean	3.58 a	3.75 a	3.5 a	3.33 a	3.33 a	

*Means with same letters lack a significant difference at 5% level by Duncan's test

Table 5. The effect of Zn and B on the Cu uptake (kg ha^{-1}) by the grain*

B (kg ha^{-1})	Zn (kg ha^{-1})					
	0	8	16	24	Foliar Spray	Mean
0	22.53 e	39.6 ab	30.5 bcde	26.43 cde	26.4 cde	29.09 a
3	32.93 abcde	38.57 abc	36.3 abcd	24.93 de	31.8 bcde	32.91 a
6	26.1 cde	29.13 bcde	25.9 de	24.43 de	33.17 abcde	27.75 a
Foliar Spray	25.17 de	23.13 e	32.6 abcde	44.57 a	24.23 de	29.94 a
Mean	26.68 a	32.61 a	31.33 a	30.09 a	28.9 a	

*Means with same letters lack a significant difference at 5% level by Duncan's test

3.6. Correlation between the concentration and total uptake of Cu and N in grain with other variables

Concentration and uptake and other variables, correlation coefficients (R) and (R^2) between different variables were computed using the Pearson method and equations relating to each variable were derived using the step-by-step method. The symbols * and ** in equations denote significance at 5 percent level ($\alpha = 0.05$) and 1 percent level ($\alpha = 0.01$) respectively.

3.6.1. Nitrogen concentration in the grain

The grain N content showed a positive correlation with the leaf K content (R= 0.38), Fe (R= 0.32), Zn (R= 0.31) and B (R= 0.47*), the grain B content (R= 0.43), the uptake of N (R= 0.67**) and B (R= 0.42) by the grain, the percentage of grain in the ear (R= 0.40) and grain protein content (R= 0.99**) and a negative correlation with the leaf N content (R= -0.38) and P (R= -0.33), the grain Cu content (R= -0.32), the ear length (R= -0.33), the ear diameter (R= -0.40) and 1000-grain weight (R= -0.38). The equations of which were:

1) NG = - 0.00144 + 0.176 P R = 1**

2) NG = - 0.00186 + 0.176 P – 0.00000945 BUG R = 1**

3) NG = 0.00408 + 0.176 P – 0.0000143 BUG + 0.0000496 NGL R = 1**

NG, P, BUG and NGL are N grain content (%), grain protein content (%), B uptake by the grain (g ha^{-1}) and number of grains in the ear length, respectively.

3.6.2. Nitrogen uptake by the grain

There was a positive correlation between N uptake by the grain and the leaf Zn content (0.41), the grain N content (0.67**), P content (R= 0.33), Mn content (0.38) and B content (0.53*), the uptake of P

(0.79**), K (0.78**), Fe (0.31), Mn (0.63**), Zn (0.56**), Cu (0.31) and B (0.76**), ear weight (0.68**),), grain weigh in the ear (0.69**), total grain yield (0.88**), the number of grains in the ear length (0.58**), the number of grains across the ear diameter (0.31), grain protein content (0.67**), and a negative correlation with leaf Mn content (-0.32) and Cu content (-0.33). The equations of which were:

1) NUG = -22.417 + 0.0197 TGY R = 0.882**
2) NUG = -145.955 + 0.017 TGY + 86.073 NG R^2 = 0.998**
3) NUG = -146.408 + 0.0171 TGY + 83.816 NG + 4.847 CuS R^2 = 0.999**
4) NUG = -139.577 + 0.0172 TGY + 81.666 NG + 6.573 CuS – 0.000374 DM R^2 = 0.999**

NUG, TGY, NG, CuS and DM denote N uptake by the grain (kg ha^{-1}), total grain yield (kg ha^{-1}), grain N content (%), soil Cu content after harvest (mg kg^{-1}) and dry matter (kg ha^{-1}), respectively.

3.6.3. Copper concentration in the grain

The grain Cu content showed a positive correlation with the leaf P content (R = 0.39), Cu uptake by the grain (R = 0.85**), and a negative correlation with the percentage of grain in the ear (R = -0.30) and grain protein content (R = -0.32). The equations of which were:

1) CuG = 1.063 + 0.0814 CuUG R = 0.854**
2) CuG = 3.624 + 0.114 CuUG – 0.000412 TGY R^2 = 0.988**
3) CuG = 3.089 + 0.117 CuUG – 0.000436 TGY + 0.0667 P R^2 = 0.991**
4) CuG = 3.193 + 0.116 CuUG – 0.000403 TGY + 0.0726 P – 0.00198 GW R^2 = 0.993**

CuG, CuUG, TGY, P and GW are grain Cu content (mg kg^{-1}), Cu uptake by the grain (g ha^{-1}), total grain yield (kg ha^{-1}), grain protein content (%) and grain weight in the ear (g), respectively.

3.6.4. Copper uptake by the grain

There was a positive correlation between Cu uptake by the grain and with the leaf P content (R = 0.46*), the grain Cu content (R = 0.85**), the uptake of N (R = 0.31), P (R = 0.36), Mn (R = 0.41), and Zn (R = 0.39) by the grain, ear weight (R = 0.33), the total grain yield (R = 0.55**), the number of grains along the ear (R = 0.44**), the number of grains across the ear diameter (R = 0.43), and a negative correlation with leaf Fe content (R =-0.53) and grain K content (R = -0.40). The equations of which were:

1) CuUG = -1.421 + 8.955 CuG R = 0.854**
2) CuUG = -31.551 + 8.695 CuG + 0.00363 TGY R^2 = 0.992**
3) CuUG = -25.995 + 8.484 CuG + 0.00374TGY – 0.596 P R^2 = 0.995**
4) CuUG = -27.144 + 8.553 CuG + 0.00348 TGY – 0.642 P + 0.167 GW R^2 = 0.996**

CuUG, CuG, TGY, P and GW are Cu uptake by the grain (g ha^{-1}), grain Cu content (mg kg^{-1}), total grain yield (kg ha^{-1}), grain protein content (%) and grain weight in the ear (g), respectively.

4. Conclusion

Application of Zn to the soil and spraying it increased N uptake in the grain. The least and the highest N uptake in the grain at 125.99 and 155.92 kg ha^{-1}, were seen at no Zn level and 8 kg ha^{-1} Zn level, respectively. Also application of B at all levels increased N uptake in the grain. The least and the highest N uptake in the grain at 129.07 and 155.26 kg ha^{-1}, were seen at no B level and B spraying level, respectively. Boron spraying at 8 kg ha^{-1} Zn level decreased Cu concentration and uptake in the grain; but at 24 kg ha^{-1} Zn level, increased Cu uptake in the grain. Zinc application had no effect on Cu concentration in the grain at any B levels. Application of 8 kg ha^{-1} Zn at zero B level, and 24 kg ha^{-1} Zn at B spraying level, increased Cu uptake in the grain.

ACKNOWLEDGMENTS

I would like to thanks my father for assistance in this research project.

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12/12/2010

Using Nonwoven Hollow Fibers to Improve Cars Interior Acoustic Properties

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Abstract: The necessity and importance of protecting our environment is evident in this time and the high developed industrial nations are responsible in the first position The industrial fabrics industry will continue to develop to meet the needs of society and the growth of the industry is assured because industrial fabrics are leading the way for materials to be structured to solve problems and to be engineered to meet the special performance of products. In the past few years, car manufacturers have focused on automobile interiors from a merely function as not only do interior trims serve to differentiate models, but materials and designs can also be used to tailor the same model to different target groups. This research aims to produce nonwoven fabrics that can be used in car interior components (head liners, doors, side panels and trunk liners) to prevent noise from reaching the passenger compartment and so achieving comfort in the car interior. Two kinds of fibers were used polyester and hollow polyester fibers, both of 6 denier, to produce three different fabrics of 100 % polyester fibers, 75 % polyester /25 % hollow polyester fibers, and 55 % polyester /45 % hollow polyester fibers. Four fabric weights were produced 300, 400, 500 and 600 g/m^2. All samples were bonded using thermal bonding technique. More results were reached, for examples, samples produced with high percentage of hollow fibers had recorded the highest rates of sound absorption, whereas samples produced with 100% polyester fibers have recorded the lowest rates. It was also found that there are direct relationship between weight per m^2 and sound absorption efficiency. All samples have achieved the expected results and sample produced with 55% polyester/45% hollow polyester fibers and 600 g/ m^2, have achieved the best results.
[Abdelfattah, A. Mahmoud, Ghalia E. Ibrahim and Eman R. Mahmoud. **Using Nonwoven Hollow Fibers to Improve Cars Interior Acoustic Properties.** Life Science Journal. 2011;8(1):344-351] (ISSN:1097–8135). http://www.lifesciencesite.com.

Keywords: Nonwoven fabrics, Hollow Fibers, Cars Interior, Acoustic properties, sound insulation.

1. Introduction:

When talking about textiles most people think of clothing, home textile and the like; only few think about the automotive industry [1]. In fact automotive textiles are considered one of the most important markets in the technical textiles sector. Over the last decade [2], the field of non-conventional textiles has been witnessing a material revolution which has resulted in improved and economical products [3]. The automotive industry has become so competitive that manufactures are reluctant to divulge precise details of their process for fear that it could be helpful to their competitors .Industrial textiles are widely used in transportation vehicles and systems including cars, trains, buses, airplanes and marine vehicle [4], automotive textiles are growth markets in terms of quantity, quality, and product variety [5] The motor vehicle remains an important means for individual transport worldwide. The interior of transportation vehicles are receiving more attention these days. Acoustical insulation products are frequently used in automotive interiors to reduce heat levels [6]. That customers expect more comfort, better safety, good appearance, (both externally and internally), high performance and good fuel mileage [2]. Textiles make a major contribution towards realizing all these expectations due to its advantages which made it a preferable material, such as high capacity to take moisture, adjustable porosity, high – pile fibrous surface, low cost recycling and a wide range of ways to combine textiles with other materials.[7]. Due to the diverse product range, automotive textiles can be classed into upholstery, carpeting [8], which have a decisive influence on the acoustic comfort [9], interior components such as head liners, and doors and side panels [8], which are foam backed components to achieve easier installation and improve acoustical properties) [10] tires, safety devices (such as seat belts and airbags), filters and engine compartment items [8].

1-1. Noise in cars

Noise has become serious environment pollution in our daily life and is an increasing public health problem, as noise can cause serious health effects such as hearing loss, sleep disturbance, tiredness, cardiovascular and psychophysiology problems and performance reduction. Therefore it is very important to control or reduce noise from traffic, and in factories, offices and houses [11]. Car noise is

essentially caused by the unit sound, the exhaust system noise, the air suction, noise, rolling and wind noises [12]. Nowadays dominant approach to achieving interior quietness relies to a large extent upon the ability to create impermeable enclosures around vehicle occupants through the use of several heavy interior layers (sound transmission loss), but recently a new concept has been emerged suggesting that sound can be reduced by replacing reflection (sound transmission loss) with dissipation (interior sound absorption area) by eliminating heavy barrier layers with light weight porous materials [13].

Nonwoven is employed as fabrics for different kinds of interior applications. The numerous applications of nonwoven in cars can be classified into functional or aesthetic but there is a third category that of substitutes for other materials. Nonwoven can be made in a wide range of densities and different forms, the use of nonwoven is increasing because it offers great versatility and cost effectiveness [14]. Each vehicle requires about 20 m^2 of nonwoven materials [11], which are used especially for insulation, noise dissipation and as filter materials ….etc. Woven and knitted fabrics are also used in producing automotive fabrics but to a lesser extent compared to nonwoven fabrics [15].

The present work is undertaken with a view to produce nonwoven fabrics which can be used to induce comfort in the car interior. By virtue of their ability to prevent noise, there fabrics can be safely used to achieve comfort in the passenger compartment as a manifestation of preventing noise from reducing this compartment. The nonwoven fabrics are made of polyester and hollow polyester at different ratios and weights.

2. The experimental work

2-1. Specification of samples under study

In order to produce samples under study nonwoven technique was applied using cross-laid fiber orientation. Two kinds of textile materials were used polyester and hollow polyester fibers, both of 6 denier, to produce three different fabrics, of 100 % polyester fibers, 75 % polyester/ 25 % hollow polyester fibers and 55 % polyester/ 45 % hollow polyester fibers. Four fabric weights were also produced 300, 400, 500 and 600 g/m^2. All samples were bonded using thermal bonding technique (hot air method (by adding a small proportion of low–melting point polyester fibers (about 15 % and melting point 110^0c). Tables (1) and (2) illustrate the specification of all samples produced.

2-2. Hollow fibers

Hollow fibers are polymeric fibers that have a continuous hole running down the middle; the hole is created by the introduction of air or other gas (nitrogen) in the polymeric solution (in wet spinning process) or by melt spinning through specially designed spinnerets [16]. Hollow fibers provide greater bulk with less weight. They are, therefore often used to make insulation fabrics [17].

2-3. Tests applied to samples under study

In order to evaluate the performance properties of the produced samples, the following tests were carried out:-

1-Sound absorption coefficient test was carried out according to the ASTM-E 1050-1982 [18]. The sound (noise)absorption values (%)of samples under study were measured at 6 different frequencies,125,250,500,1000,2000, and 4000Hz.

2-Air permeability test was carried out according to the (ASTM-D 4491-92) [19].

3-Fabric thickness test was carried out according to the (ASTM D 1777) [20].

3. Results and discussion:

Table (1), shows the production specification of the samples under study; where as table (2), illustrates the specifications of three samples. On the other hand, dependence of air permeability on fabric thickness is shown in table (3).

Statistical analysis of the data were made with relationships between variables. Regression equation and correlation coefficient for the effect of fiber type on fabric sound absorption at weights of 300, 400 and 500 g/m^2 are set out in tables (4), (5) and (6), respectively.

On the other hand, tables (7), (8) and (9) depict the regression equation and correlation coefficient for the effect of weight g/m^2 on fabric sound absorption when the fabrics were made of 100% polyester, 75% polyester/25% hollow fiber and 55% polyester/45% hollow fiber, respectively.

In addition to the above, table (10) discloses the regression equation and correlation coefficient for the effect of weight g/m^2 and fiber type on fabric airpermeability. Meanwhile, table (11) reveals the regression equation and correlation coefficient for the effect of weight g/m^2 and fiber type on fabric thickness.

3.1 Sound absorption efficiency test

Figures from (1 to 4), signify that there is a direct relation between the increase in hollow fibers percentage in the fabric and its sound absorption efficiency. This is most probably due to the hollow structure of the fibers. Such structure would have air inside its lumen which increases the air volume in the fabric and, in turn, increases its ability to absorb sound waves rather than reflecting it.

Figures from (5 to 7), disclose that, the increase in fabric weight improves the sound absorption efficiency of fabrics at both low and high frequencies. We can state that this is mainly, because the increase in fabric weight means an increasing in the number of fibers per unit area and also an increase in fabric volume, and so the interconnected voids will be increased which absorb the sound waves rather than reflecting it, if the fabric was compacted, which means that the sound absorption efficiency will be increased.

3.2 Air permeability test

From table (3) and figure (8), it can be seen that there is an inverse relationship between fabric weight and its air permeability properties. We can state that the increase in fabric weight increases number of fibers per unit area and so free spaces in fabric will be decreased which delay the free passage of air through the fabric. Besides this the effect of heat bonding technique helps in this result because of the melting of fibers (polyester fibers of low melting point) which cause pore spaces of free area between fibers to be decreased leading to the decrease in fabric air permeability. It can also be noticed from figure (8) that, for the same weight, the more hollow fibers the less air permeability of the fabrics become due to the increase in number of hollow fibers, which leads to increase of the air volume in the fabric, because of the air entrapped into the fibers. Accordingly the free spaces into the fabrics will be decreased because of fabric bulkiness and so the ability of air flow to be passed through the fabric will be also decreased.

3.3 Fabric thickness test

It is obvious from table (3) and figure (9) that there is a direct relationship between fabric thickness and its weight. We can report that, this is because of the fact that the increase in fabric weight means an increase in number of fibers per unit area which leads to the increase in fabric thickness. It is also clear from table (3) and figure (9) that the increase in hollow fibers percentage in the fabric increases its thickness. This might to due to the structure of hollow fibers which have air in its internal voids which increases the volume ratio of fibers, leading to the increase in fabric bulkiness and also its thickness.

Table (1) specification of samples production

Sample No.	**Property**	**Specification**
1	Fiber type	Polyester and hollow polyester fibers
2	Fiber count	6 denier
3	Fiber length	64 mm
4	Fabrics material	100 % polyester fibers ,75 %polyester /25 % hollow polyester fibers and 55 % polyester / 45 % hollow polyester fibers.
5	Web formation	Cross-laid
6	Fabric weight (g/m^2)	300,400,500 and 600
7	Bonding technique	Thermal bonding

Table (2) specification of all samples under study

Sample No.	**Fabric weight g/m^2**	**Fabric material**
1	300	100 % polyester fibers
2	300	75 % polyester /25 % hollow polyester fibers
3	300	55 % polyester /45 % hollow polyester fiber
4	400	100 % polyester fibers
5	400	75 % polyester /25 % hollow polyester fibers
6	400	55 % polyester /45 % hollow polyester fiber
7	500	100 % polyester fibers
8	500	75 % polyester /25 % hollow polyester fibers
9	500	55 % polyester /45 % hollow polyester fiber
10	600	100 % polyester fibers
11	600	75 % polyester /25 % hollow polyester fibers
12	600	55 % polyester /45 % hollow polyester fiber

Table (3) results of Air permeability test.

Sample No.	Thickness (mm)	Air permeability (cm^3/ cm^2/sec)
1	3.3	212
2	4.5	196
3	4.9	195
4	6.1	195
5	7.7	182
6	7.8	160
7	9.8	152
8	9.9	148
9	10.1	129
10	11.4	127
11	13.6	122
12	15	108

Table (4) regression equation and correlation coefficient for the effect of fiber type on fabric sound absorption, at weight 300 g/m^2

Fiber type	Regression equation	Correlation coefficient
100%Polyester	Y=9.94247 X +0.0372916	0.952573722
75%Polyester /25%hollow fiber	Y=0.0001303 X +0.036958	0.958470874
55%Polyester /45%hollow fiber	Y=0.0001382 X +0.038416	0.95657395

Table (5) regression equation and correlation coefficient for the effect of fiber type on fabric sound absorption, at weight 400 g/m^2

Fiber type	Regression equation	Correlation coefficient
100%Polyester	Y=7.78871 X +0.027875	0.97704865
75%Polyester /25%hollow fiber	Y=0.0001233 X +0.029083	0.96701712
55%Polyester /45%hollow fiber	Y=0.0001055 X +0.033416	0.9624999

Table (6) regression equation and correlation coefficient for the effect of fiber type on fabric sound absorption, at weight 500 g/m^2.

Fiber type	Regression equation	Correlation coefficient
100%Polyester	Y=0.00013317 X +0.039376	0.95556476
75%Polyester /25%hollow fiber	Y=0.0001495 X +0.0420416	0.954627931
55%Polyester /45%hollow fiber	Y=0.000166 X +0.0345	0.961916708

Table (7) regression equation and correlation coefficient for the effect of weight g/m^2 on fabric sound absorption, at polyester 100 %.

Weight /m^2	Regression equation	Correlation coefficient
300	Y=8.1626 X +0.17199	0.976762
400	Y=0.0001044 X +0.02288	0.957373368
500	Y=0.0001387 X +0.023338	0.9609214
600	Y=0.000178 X +0.0246766	0.96088684

Table (8) regression equation and correlation coefficient for the effect of weight g/m^2 on fabric sound absorption, at 75 % polyester/25 % hollow fiber.

Weight /m^2	Regression equation	Correlation coefficient
300	Y=0.000127784 X +0.0162835	0.9706377
400	Y=0.000143 X +0.022208	0.9618994
500	Y=0.000172321 X +0.186616	0.966878
600	Y=0.000127784 X +0.016283	0.9706377

Table (9) regression equation and correlation coefficient for the effect of weight g/m^2 on fabrics sound absorption, at 55%polyester/45%hollow fiber .

Weight /m^2	Regression equation	Correlation coefficient
300	Y=0.00011024 X +0.01996517	0.9660334
400	Y=0.00013567 X +0.02176619	0.963322316
500	Y=0.00015565 X +0.02454228	0.960252268
600	Y=0.0001956 X +0.03019403	0.952865124

Table (10) regression equation and correlation coefficient for the effect of weight g/m^2 and fiber type on fabric air permeability.

Fiber type	Regression equation	Correlation coefficient
100%Polyester	Y=-0. 34 X +328	0.9885229
75%Polyester /25%hollow fiber	Y=-0.26 X +262.3333	-0.9938927
55%Polyester /45%hollow fiber	Y=-0.3 X +300.666	0.997050141

Table (11) regression equation and correlation coefficient for the effect of weight g/m^2 and fiber type on fabric thickness.

Fiber type	Regression equation	Correlation coefficient
100%Polyester	Y=0. 28 X +4.95	0.989825773
75%Polyester /25%hollow fiber	Y=0.0295X + 4.36	0.995418547
55%Polyester /45%hollow fiber	Y=-0.36 X -7.03333	0.9789502

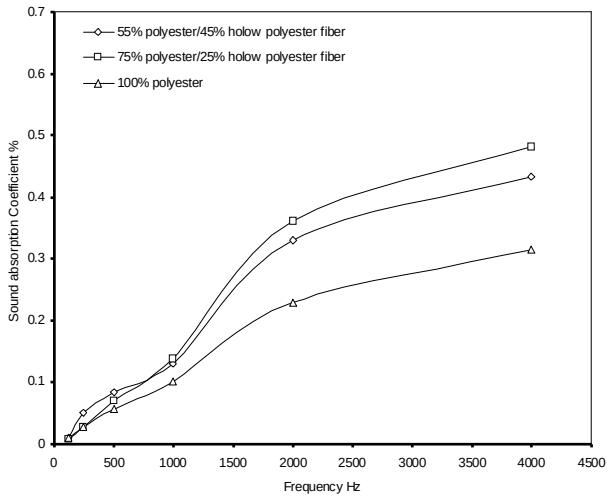

Fig.1, Effect of fiber type on fabric sound absorption at weight 300g/m^2

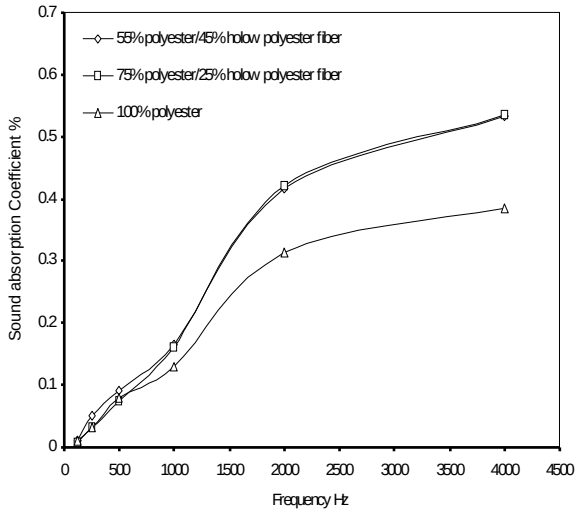

Fig.2, Effect of fiber type on fabric sound absorption at weight 400g/m^2

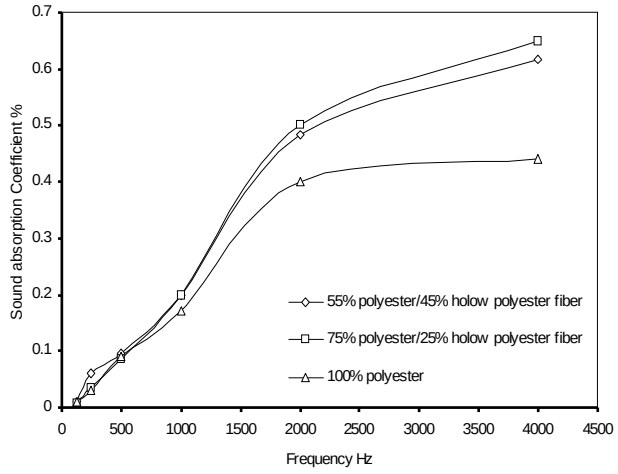

Fig.3, Effect of fiber type on fabric sound absorption at weight 500g/m^2

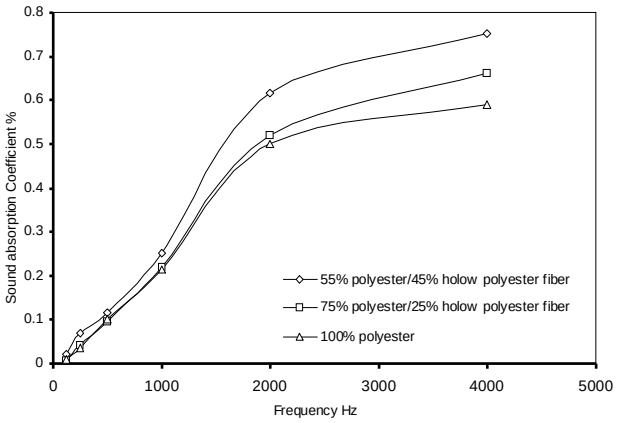

Fig.4, Effect of fiber type on fabric sound absorption at weight 600g/m^2

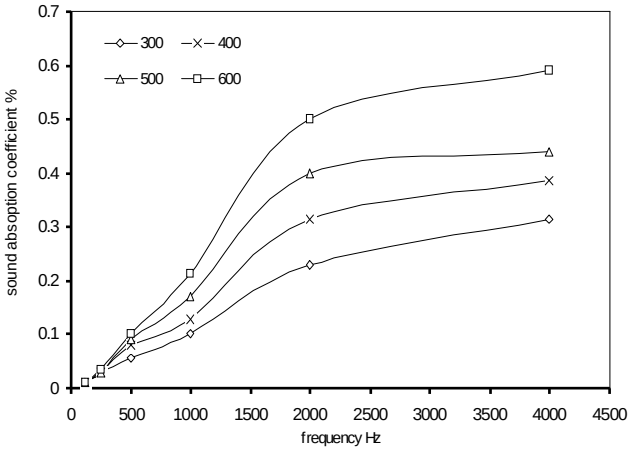

Fig.5, Effect of weight /m^2 on fabric sound absorption at 100%polyester

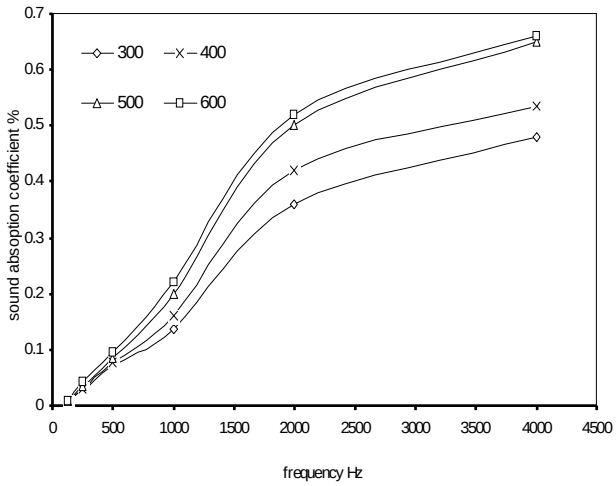

Fig.6, Effect of weight /m^2 on fabric sound absorption at 75%polyester/ 25% hollow fiber

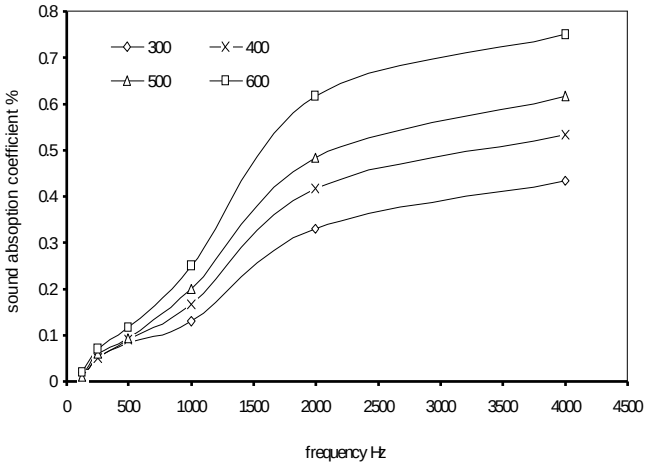

Fig.7, Effect of weight /m^2 on fabric sound absorption at 55%polyester/ 45% hollow fiber

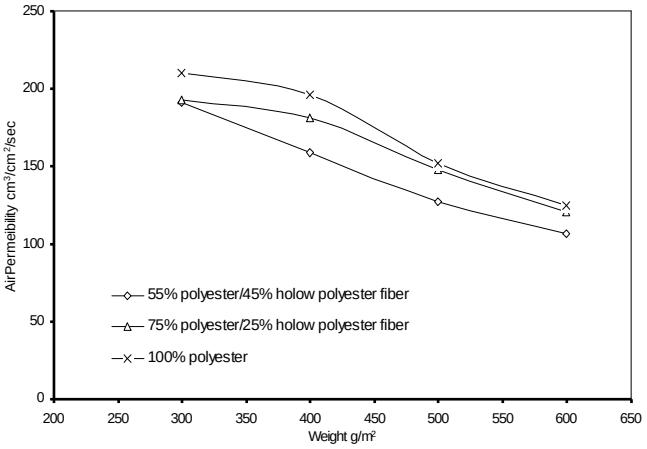

Fig.8, Effect of weight /m^2 and fiber type on fabric Air Permeability

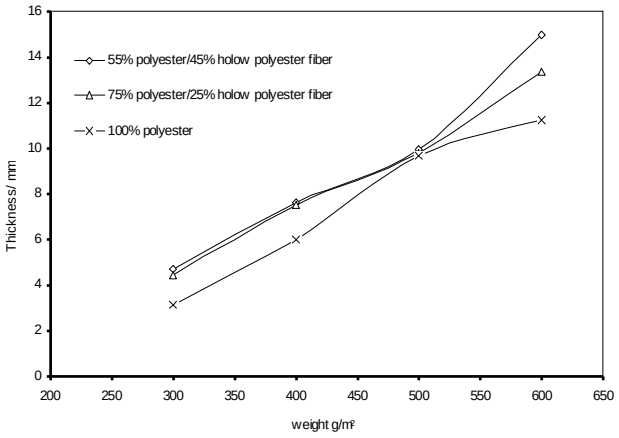

Fig.9, Effect of weight /m^2 and fiber type on fabric thickness

4. Conclusion

Over the past decade improvements in both functional and appearance durability of automotive trim have been significant. Technology applied in the form of better polymers, stabilizers and testing procedures has contributed to these improvements. Fabrics provide comfort and better appearance in car interiors while still meeting the performance and consistency requirements, these fabrics must also meet the needs of fashion function and durability required by the automakers. In the present research trials were made to produce fabric for sound insulation in automotive. Thus fabrics were developed for use in car interior components to minimize noise from reaching the passenger compartment and so achieving comfort in the car interior. Two kinds of fibers were used polyester and hollow polyester fibers, both of 6 denier, to produce three different fabrics of 100 % polyester fibers, 75 % polyester /25 % hollow polyester fibers, and 55 % polyester/45 % hollow polyester fibers. Four fabric weights were produced 300, 400, 500 and 600 g/m^2. All samples were bonded using thermal bonding technique. More results were reached, for examples, samples produced with high percentage of hollow fibers had recorded the highest rate of sound absorption, and samples produced with 100% polyester fibers have recorded the lowest rates. It was also found that there is direct relationship between weight per m^2 and sound absorption efficiency. Similarly samples produced with high percentage of hollow fibers recorded the highest rates of sound absorption, whereas samples produced with 100% polyester fibers recorded the lowest rates. Sample produced using 55% polyester/45% hollow polyester fibers and 600 g/m^2, displayed the best results.

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12/28/2010

Application Participatory Rural Communication Appraisal (PRCA) A New Tool for Rural Development (Case Study Khouzestan Province, Iran)

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Abstract: Participatory Rural Communication Appraisal (PRCA) is suitable tool to identify, prioritize and analyze of needs and problems of the people, while opportunities and solutions existing in the community are discovered. PRCA specifically seeks to discover issues to resolution through the application of communication. The method of this research was qualitative research with semi structure interview. At this research 5 analytical loops organized. In each loop one facilitator person and 6 to 9 farmers as analyzer were exist. By using different PRCA techniques such as matrix, community map, cause and effect diagram, factor analysis diagram, tree diagram etc, identified educational needs, situation geographical and natural resources and cause of environmental destruction based on farmers view. Application Participatory Rural Communication Appraisal (PRCA) A New Tool for Rural Development (Case Study Khouzestan Province, Iran).
[Ahmad Reza Ommani. **Application Participatory Rural Communication Appraisal (PRCA) A New Tool for Rural Development (Case Study Khouzestan Province, Iran).** Life Science Journal. 2011;8(1):352-355] (ISSN:1097–8135). http://www.lifesciencesite.com.

Keywords: PRCA, Rural Development, Watershed

1. Introduction

Participatory Rural Communication Appraisal (PRCA) is a communication research method that use visualization techniques and interviews to create information for the design of effective communication programs, materials, media and methods for development purposes to ensure relevance and ownership by the farmers. PRCA facilitates conversation among the rural people themselves and between them and the extension agents in order for all parties to reach mutual understanding and plan for action. PRCA is therefore used to promote the participation of rural people in decision-making that affects their living (Anyaegbunam et al, 2004). PRCA and Participatory Rural Appraisal (PRA) are tools to increase participation and communication by local people organizing for rural and agricultural development (Toness, 2001). PRA is 'a growing family of approaches and methods to enable local people to share, enhance and analyse their knowledge of life and conditions, to plan and to act' (Chambers, 1994: 1). In last decades Participatory Rural Appraisal (PRA) and later Participatory Learning and Action (PLA) methods emerged. PRA and PLA recognized that there were many things that researchers and subject matter specialists did not know and the only way to know them was by listening to the rural people. Similarly rural people were lacking some of the technical knowledge that the experts had to solve some of their problems. Thus, knowledge sharing became an essential component of PRA (Noorivandi and Ommani, 2009., Anyaegbunam et al, 2004).

PRA has been used extensively in agriculture, forestry and a number of other areas. PRCA belongs to the same family as PRA, PLA and the other participatory methods, but it is unique because it focuses specifically on rural communication systems and how to improve information sharing among all rural people in a development effort. From the time it was conceptualized in 1994, PRCA has undergone changes to better adapt it to field realities. (Anyaegbunam et al, 2004).

Tools and techniques used for PRCA are mainly take on from other participatory appraisal approaches such as PRA, PLA, etc. Since most of these tools and techniques are visual in nature, they remove the need for high levels of literacy and numeracy on the part of community members. The primary purpose of PRCA tools and techniques is to enable groups in the community to express and analyze their knowledge and needs. They help the people to map and diagram their situation and environment in the most easy and non-threatening manner using materials and symbols that they are used to. The tools and techniques also assist the people to easily identify and prioritize their needs, opportunities, problems, strengths, weaknesses and threats(Anyaegbunam et al, 2004, p.64).

2. Material and Methods

The application of PRCA techniques (Case Study: rural area in Shoushtar township of Khouzestan provinces)

The research method is qualitative that carry out by 5 analytical loop in rural area of Shoushtar

township of Khouzestan province, Iran. PRCA assist the community to set their own qualitative indicators for participatory monitoring and evaluation of the situation. This set of indicators is defined by the community and often reflects measures of their satisfaction, viewpoint or perception regarding educational needs, their situation, or analyzing of programs. There is a different technique for applying PRCA based on purpose of research:

1. Techniques for identify and describe of community

1.1. Techniques for gathering data regarding geographical location: Participatory mapping allows a group of people share information regarding location. Community maps apply to visually define of farms, water resources, gardens etc.

1.2. Techniques for gathering data regarding residential and organizational location: Residential and location maps show status of home location, schools, organizations, infrastructure etc (Figure 1).

2. Techniques for educational need assessment of people.

2.1. Matrix one of techniques for identifying educational needs is classification matrices table. This matrix that has both horizontal and vertical axis. A wide range of matrices can be constructed using local materials, giving scores for different variables, such as the productivity of particular crop varieties or methods of soil and water conservation. Labor inputs, taste preference or fertilizer use, for example, might be plotted against particular rice or millet varieties. At this research matrix used for determining educational needs of Garab farmers area in Shoushtar township of Khouzestan province, Iran. By one analytic loop with one facilitator (researcher) and farmers, determined educational needs of Garab farmers regarding watershed practices. By perception of farmers this need ranked (Figure 2).

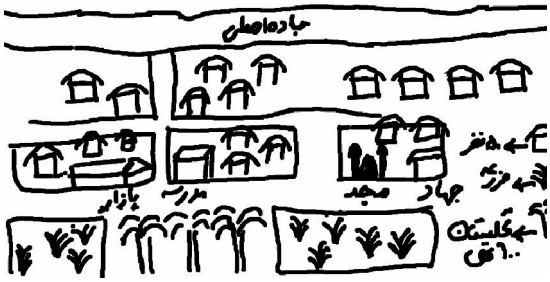

Figure 1: Examples of map community and organizations, designed by farmers of rural area in Shoushtar township of Khouzestan provinces, Iran

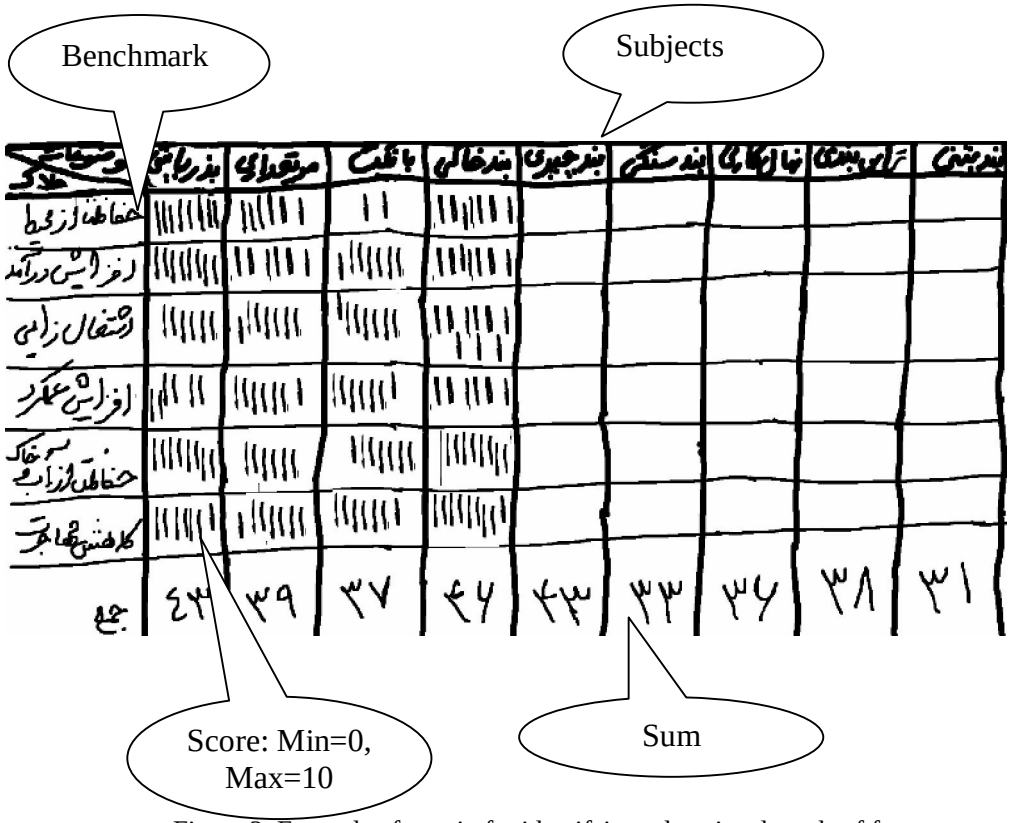

Figure 2: Example of matrix for identifying educational needs of farmers

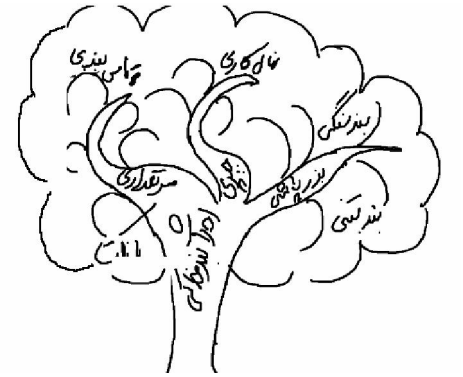

Figure 3: Example of tree technique for identifying educational needs

2.2. Tree design: one of other techniques for identifying educational needs is tree design. Stem of tree was considered for main educational need. On other cluster write other need with lower important (Figure 3).

3. Techniques for Identifying Cause of Matters

3.1 Cause and effect diagram is one of techniques for visual comprehensive regarding cause of different matters. At this research the cause of distraction and depletion of pastures and lands analyzed. Based on results, reduction of plants, machinery, animals, chemical inputs, deep tillage etc, are main cause of them(Figure 4).

3.2 Factor analysis diagram is other techniques for determining factors that affect on other variables. At this research analyzed different variable and identified the factors that affect on conservation natural resources base on view of farmers (Figure 5). Same variable considered as one group. Based on view of farmers 3 groups categorized: people supports, governmental supports and extension activities.

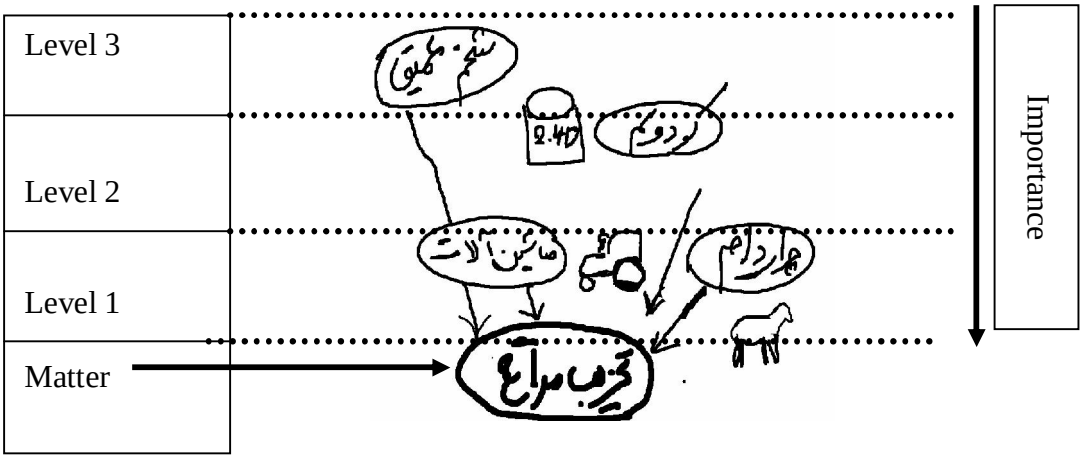

Figure 3: Example cause and effect diagram for identifying cause of distraction and depletion of pastures and lands

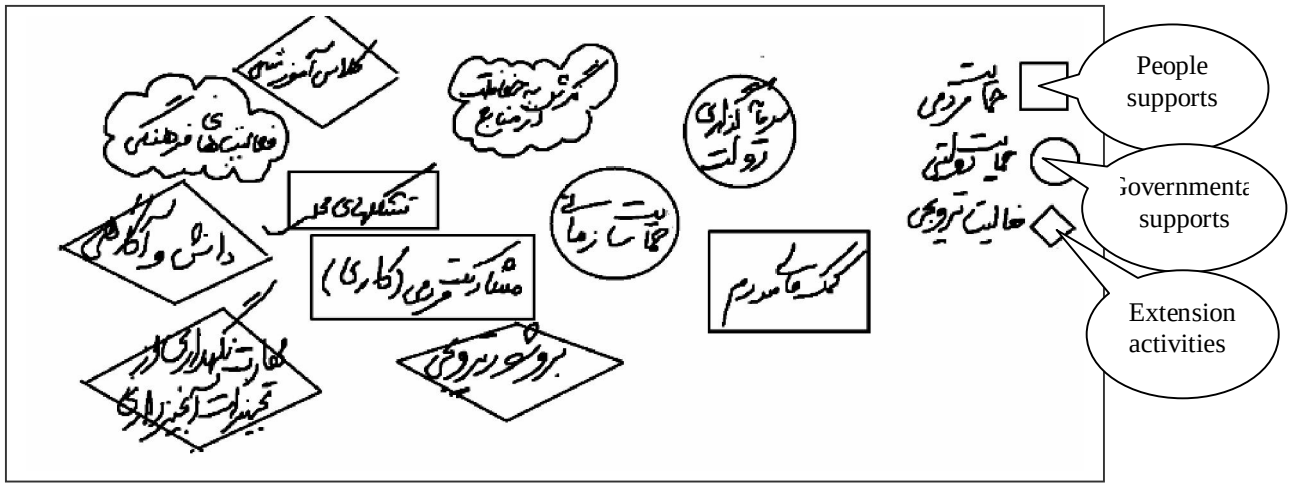

Figure 3: Example of factor analysis diagram for identifying the factors that affect on conservation natural resources

Conclusion

Through PRCA, rural people express and share what they already know, investigate and observe add to their knowledge, analyze and become more aware and reach new understanding plan and implement what they have planned take command, and further learn through the experience of action. The participatory orientation of PRCA has given new impetus to the development of methods. Based on different researcher PRCA are good for (Adebo, 2000):

• Providing basic information in situations where little in known

• Identifying and assessing problems

• Appraising, designing, implementing, monitoring, and evaluation programs and projects

• Getting a better picture of needs and organizations' ability to meet them

• Developing and transferring appropriate technologies

• Appraising emergencies

• Planning projects that are more relevant, restructuring administrations, assisting in decision-making and policy formation

• Generating hypotheses, ruling out inappropriate ones

• Providing guidelines for survey designs and assessing the applicability of their results to other places.

• Fleshing – out complementing, interpreting, or giving depth and context to information obtained through other methods.

At this research described application of some PRCA techniques on empowerment of farmers. PRCA techniques used for:

- How many people living in community
- How they living and working
- Identifying educational need assessment
- Identifying situation geographical and natural resources.
- Techniques for identifying cause of matters

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1/7/2010

Study on Variation Effects Caused by Ion Beam-mediated Transformation Whose Transformation Receptors are Wheat's Segregation Population Seeds

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Abstract: To provide theory evidence for ion beam transformation used in traditional breeding easily, the variation effects were studied that wheat's segregating population seeds were used as transformation receptor via ion beam implantation. Wheat's F2 segregating population seeds of two hybridization combination were used as transformation receptor via ion beam implantation, and the genome DNA of Hongmang wheat and Hexaploid Triticale were used as transformation donor.The result showed that the germination rate of different transformation combination was different. The coefficient variability of spike length of wheat main axic increased significantly. The average of plant height had decreasing trend. Both average and coefficient variability of grain's quality characters had decreasing trend. All the above results show that after doing ion beam-mediated transformation to segregation population seed, the coefficient variability of some characters could increase significantly, in another word, ion beam-mediated transformation could widen the variation spectrum of some characters.
[Gu Yunhong, Gao Fei, Qi Yanlei, Jiao Zhen. Study on Variation Effects Caused by Ion Beam-mediated Transformation Whose Transformation Receptor is Wheat's Segregation Population Seed. Life Science Journal. 2011;8(1):356-362] (ISSN:1097–8135). http://www.lifesciencesite.com.

Key words: Transformation mediated by ion beam implantation; Wheat; Segregating population

1. Introduction

Transformation technique has combined with conventional breeding technique and applied in wheat genetic improvement. It could improve the actuality that wheat's genetic basis is strait, genomic resources are lack. Now, the main wheat genetic transformation methods are particle bombardment (Takumi et al., 1994, Chugh and Khurana, 2003, TANG et al., 2006), pollen-tube pathway (LIU et al., 2005, YIN et al., 2004), agrobacterium-mediated transformation (Supartana et al., 2006, Bi et al., 2006, Haliloglu and Baenziger, 2003), et al. Since Yu reported the successful GUS and CAT gene transfer into suspension cells and mature rice embryos following the 20–30 keV argon ion bombardment (Yu et al, 1993), a number of reports have emerged on ion bombardment which induced foreign molecule transfering into plant tissues and bacteriacells (Yang et al, 1994, Li et al, 2001, Wu et al, 2000, Wu et al, 2000) . It showed special characters in wheat's transformation. The chitinasegene (RCH8) in plasmid vector pCAMBIA1308 had been reported to be delivered into three wheat cultivars (Yangmai158, Wan9210, Wanmai32) by a Low energy Ar^+ beam-mediated method, and molecular blotting assays confirm stable integration of alien DNA fragments into wheat genome (Wu et al, 2000). The plant transformation frequencies which depend on the plant species and ion fluence ranged from 0.5 to 3.8%. Jiao et al. (2006) reported wheat was transformed with soybean DNA through ion beam mediation and its high-protein offspring plants were obtained through field selections and protein determinations in the four consecutive generations. The application of ion beam-mediated transformation on wheat, especially transferring foreign genomic DNA into wheat, indicated that the merging of foreign genomic DNA could lead receptor's genomic DNA sequences' change, and could enrich wheat's genetic resources.

In present, ion beam-mediated transformation process is that put stable varieties and strains as transformation receptor first, and then obtain transformation material via choice, and then modify and use these transformation materials. While wheat traditional breeding process is choicing single plants from F2 segregation population. To combine the dominance of ion beam-mediated transformation and traditonal breeding and improve traditional breeding via ion beam-mediated transformation, the variation effects caused by ion beam-mediated transformation which put wheat's segregation population seed as transformation receptor were studied.

2. Material and Methods

2.1 Material

2.1.1 Receptor wheat material

Two wheat crossing combinations were named combination 1(Yunong 118×Shannong 520853) and

combination 2 (Yi 12×Lankaoaizao 8).

2.1.2 Donor material

When Hongmang wheat and hexaploid triticale grew to 3-5 leaf, extracted leaf genomic DNA using SDS alkaline lysis method. The OD260/OD280 =1.89-1.95. The extracting leaf genomic DNA was used as transformation donor DNA.

2.2 Method

2.2.1 Ion beam-mediated transformation method

Seeds of F2 of each combination were divided into two groups. Each group had 500 seeds. One of the two groups was named "control group" which was not implanted by ion beam. The other was named "treatment group" which was implanted by $3\times10^{17}N^{+}/cm^{2}$. Implantation instrument was TITAN pulse implantation instrument. Frequency of pulse was 25 Hz. Width of pulse was 400μs. Energy of ions was 30keV. Type of ions was N^{+}. Flux of ion beam was 2mA. Wheat seeds were put into the holes of the sample plat one by one with embryo upwards and were implanted by ions. After the treatment group of combination 1 was implanted, these seeds were dipped into 300μg/ml genomic DNA solution of Hongmang wheat. The temperature of solution maintained at 25℃. After 12h, refreshed the DNA solution and dipped another 12h. Then the seeds of control group and treatment group were planted in field at the same time. Distant between plants was 10cm. After the treatment group of combination 2 was implanted, these seeds were dipped into 300μg/ml genomic DNA solution of hexaploid triticale wheat. The dip method was same as the above. After dip, the seeds of control and treatment group were planted in field at the same time. The condition of planting was same as the above.

2.2.2 Agronomic traits' statistication

Statistication of germination rate: 100 seeds were placed on wet filter papers in the condition of 25℃. 7 days late, germination rate was statisticated. Germination rate=the number of germinating seeds/100. The rate of survival plant was statisticated after seed-setting. The rate of survival plant=the number of plants/the number of plant seeds.The control and treatment groups were observed and noted in the whole growth period. All the single plants were pulled out after maturity and several indices, for example plant height, spike length of main axic and effective panicle number, were measured.

2.2.3 Measurement of grain quality characters

Bulk density, protein and wet gluten were measured using FOSS NIRS5000 analyzer. One day ahead of spectrum scanning, seeds were placed in the same room with the NIR analyzer in order to make seeds had same enviroment condition with analyzer. The scanning internal of wavelength was 1100-2498nm, and scanning step was 2nm. The reflect intensity(R) was collected. Each material of seeds was scanned 30 times by analyzer automatically and analyzer obtained the mean spectrum automatically. Each material was scanned 3 times repeatedly. Difference of average and coefficient variability between control and treatment group was analyzed using *t*-test and *u*-test in statistical analysis. The difference of coefficient variability could express the condition of trait segregation.

3. Result

3.1 Statistication of germination rate and survival plant rate of each transformation combination

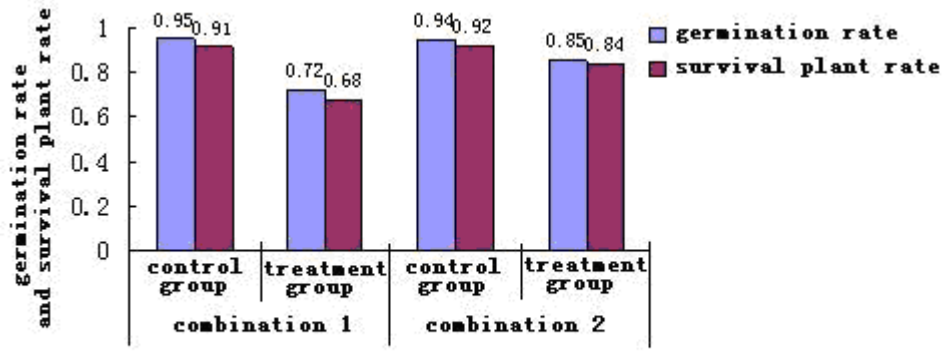

Figure 1. Germination rate and survival plant rate of different transformation combination

Transformation combination included 4 groups: control group of combination 1, treatment group of combination 1, control group of combination 2, treatment group of combination 2. Figure1 showed that survival plant rate of each group decreased little compared with germination rate. It indicated that ion beam-mediated transformation had little influence to plant growth after the seeds' germination. The germination rate of treatment group of combinatin 1 decreased by 24.2% compared with that of the control group. The germination rate of treatment group of combination 2 decreased by 9.6% compared with that of the control group. It indicated that ion beam-mediated transformation had more influence to germination rate of combinatin 1 compared with combination 2.

3.2 Agronomic traits' statistication

Table 1 showed the statistication result of plant height. To combinatin 1, the number of plants which plant height were between 70cm and 80cm in treatment group decreased from 60.0% to 18.1% compared with that of the control group, the number of plants which plant height were between 80cm to 90cm in treatment group decreased from 6.7% to 0 compared with that of the control group, the mean of plant height of treatment group decreased from 71.1cm to 63.4cm compared with that of the control group, achieved to significance level (α=0.01). Range of treatment group of combination 2 decreased from 60 cm to 47 cm compared with that of its control group, coefficient variability decreased from 21% to 16%, achieved to significance level (α=0.05).

Table 2 showed the statistication result of spike length of main axic. The mean of treatment group of combination 1 decreased to significance level (α=0.01) compared with that of the control group. The mean of treatment group of combination 2 decreased to significance level (α=0.05) compared with that of the control group, too. The coefficient variability of both combination 1 and 2 increased to significance level (α=0.05), and the result showed ion beam-mediated transformation could widen the variation spectrum of spike length of main axic.

Table 3 showed the statistication result of 1000-seed weight. The mean of treatment group of both combination 1 and 2 had no significant change compared with that of the control group. The coefficient variability of treatment group of combination 1 had no significant change compared with that of the control group, while the coefficient variability of treatment group of combination 2 decreased to significant level (α=0.05).

The maximum of effective panicle number of combination 1 and 2 appeared in treatment group. The maximum of effective panicle number of combination 1 was 26, and the maximum of effective panicle number of combination 2 was 32, while the maximum of effective panicle number of two control groups were 23.

Table 1. Statistication result of plant height

		combination 1		combination 2	
		control group	treatment group	control group	treatment group
Plant height distribu-tion range （cm）	<50(take **% of total plant number）	0.0	0.0	6.7	9.4
	50-60（as above）	5.0	23.6	29.8	18.8
	60-70（as above）	28.3	58.3	32.7	28.1
	70-80（as above）	60.0	18.1	25.0	23.4
	80-90（as above）	6.7	0.0	5.8	18.8
	>90（as above）	0.0	0.0	0.0	1.6
maximum（cm）		86	78	90	87
minimum（cm）		53	52	30	40
Range（cm）		33	26	60	47
Mean （cm±SD）		71.1±6.7	63.4±6.3**	66.8±11.0	64.2±10.3
Coefficient variability（%）		9.7	9.9	21.0	16.0*

(*significance level α=0.05,** significance level α=0.01)

Table 2. Statistication result of spike length of main axic

		combination 1		combination 2	
		control group	treatment group	control group	treatment group
Spike Length of Main Axic distribution range （cm）	5-7（take **% of total plant number）	0.0	7.0	0.0	9.6
	7-9（as above）	18.3	57.7	46.9	69.2
	9-12（as above）	81.7	35.2	53.1	21.2
maximum（cm）		11	10	11	12
minimum（cm）		7	5	7	5
Range（cm）		4	5	4	7
Mean（cm±SD）		9.3±1.0	8.0±1.1**	8.7±1.1	8.3±1.4*
Coefficient variability（%）		10.6	14.0*	12.4	16.5*

(*significance level α=0.05,** significance level α=0.01)

Table 3. Statistication result of 1000-seed weight

		combination 1		combination 2	
		control group	treatment group	control group	treatment group
1000-seed distribution range （g）	<30（take **% of total plant number）	0.0	0.0	9.4	4.8
	30-40（as above）	3.3	0.0	26.6	26.0
	40-50（as above）	36.7	45.8	32.8	50.0
	50-60（as above）	56.7	52.8	29.7	18.3
	>60（as above）	3.3	1.4	1.6	1.0
maximum（g）		63	60	60	61
minimum（g）		35	40	20	24
Range（g）		28	20	40	37
Mean（g±SD）		50.3±5.1	49.7±4.6	42.8±7.8	43.5±7.7
Coefficient variability（%）		10.3	9.3	22.7	17.7*

(*significance level α=0.05,** significance level α=0.01)

3.3 Statistication of grain quality characters

Table 4 showed the statistication result of protein content. The mean and coefficient variability of treatment group of combination 1 had no significant change compared with that of its control group. The mean and coefficient variability of treatment group of combination 2 decreased to significant level (α=0.01) compared with that of the control group.

Table 5 showed the statistication result of bulk density. The mean of treatment group of two combination had no signification change compared with that of the control group. The coefficient variability of treatment group of combination 1 decreased to significant level(α=0.05), and that of treatment group of combination 2 decreased to significant level (α=0.01).

Table 6 showed the statistication result of wet gluten. The mean and coefficient variability of treatment group of combination 1 had no significant change compared with that of the control group. The mean and coefficient variability of treatment group of combination 2 decreased to significant level (α=0.01) compared with that of its control group.

Table 4. Statistication result of protein content

		combination 1		combination 2	
		control group	treatment group	control group	treatment group
Protein content distribution range （ dry basis %）	11-13（take **% of total plant number）	5.0	0.0	6.25	14.4
	13-15（as above）	51.7	47.2	53.1	67.3
	15-17（as above）	41.7	50.0	32.8	18.3
	17-19（as above）	1.7	2.8	6.3	0
	〉20（as above）	0.0	0	1.6	0
maximum（dry basis %）		17.7	17.3	23.6	16.6
minimum（dry basis %）		11.9	13.3	12.0	11.8
Range（dry basis %）		5.8	4.0	11.6	4.8
Mean（dry basis %±SD）		14.9±1.0	15.0±1.1	14.9±1.5	14.1±0.8**
Coefficient variability（%）		7.1	7.1	12	7.3**

(*significance level α=0.05,** significance level α=0.01)

Table 5. Statistacation result of bulk density

		combination 1		combination 2	
		control group	treatment group	control group	treatment group
bulk density distribution range（g/l）	<780（take **% of total plant number）	3.3	1.4	6.3	4.2
	780-800（as above）	6.7	4.2	18.8	23.6
	800-820（as above）	25.0	23.6	35.9	50.0
	820-840（as above）	50.0	58.3	26.6	59.7
	>840（as above）	15.0	12.5	12.5	6.9
maximum（g/l）		855.8	847.9	849.5	848.1
minimum（g/l）		761.2	764.3	719.5	774.1
Range（g/l）		94.6	83.6	130	74
Mean（g/l±SD）		823.5±18.3	824.6±14.6	813±23.1	816±16.2
Coefficient variability（%）		2.3	1.8*	2.8	2.0**

(*significance level α=0.05,** significance level α=0.01)

Table 6. Statistication result of wet gluten

		combination 1		combination 2	
		control group	treatment group	control group	treatment group
wet gluten distributon range（%）	<25（take **% of total plant number）	3.3	0.0	6.25	6.7
	25-30（as above）	35.0	37.5	51.6	72.1
	30-35（as above）	58.3	54.2	37.5	20.2
	>35（as above）	3.3	8.3	4.7	1.0
maximum（%）		37.2	37.1	38.5	35.3

minimum（%）	22.3	25.1	23.1	22.5
Range（%）	14.9	12	15.4	12.8
Mean（%±SD）	30.4±2.8	30.8±2.9	29.9±3.3	28.3±2.3**
Coefficient variability（%）	9.2	9.4	14.6	8.8**

(*significance level α=0.05,** significance level α=0.01)

4. Discussion

Ion beam-mediated transformation have different influence to the germination rate of different receptor, for example the germination rate of treatment group of combination 1 decreased more compared with combination 2 (Figure 1). So before doing ion beam-mediated transformation, suitable implanting dose should be decided through previous dose experiment.

All the above results show that after doing ion beam-mediated transformation to segregation population seed, the coefficient variability of some characters could increase significantly, in another word, ion beam-mediated transformation could widen the variation spectrum of some characters. So in breeding process, both the treatment and control group of segregation population shoule be planted and choice good plants from the population, it is benefit to increase breeding efficiency.

Transformation receptor were mature seeds in this research, which is convenient to be obtained. And according to experiment object, the transformation receptor could be immature embryo, root, root tip, ovary, anther, shoot, shoot tip, stem and leaf, or callus of them.

The result showed that mean of plant height had the decreasing trend. It reached an agreement on Yang et al. (2002) and Wang et al. (1998). And at the same time, the mean of spike length of main axic had the decreasing trend, too. It had relationship with damage of ion beam implantation.

Segregation population as transformation receptor could be F2 segregation population, or other generation segregation population, for example F3, F4 and F5 generation, and could be segregation population including mixed single plants obtained from F2 and its offsprings, it could widen genotype of transformation receptor. In addition, donor gene segments accelerates gene's recombination, widens traits segregation, raises select chance and breeding efficiency.

In wheat breeding process, the choice of agronomic traits should be paid attention, and the choice of grain quality characters should be paid attention, too, for example, protein, Bulk density, wet gluten, flour yield , et al. The statistication result of grain quality characters showed, in groups which mean and coefficient variability had significant change compared with that of its control groups, the mean and coefficient variability were decreasing. Is this decreasing a universality case leading by damage of ion beam implantation or a coincidence related to the choice of transformation donor? Supposing it is the former, if every offspring is traced, do grain quality characters appear different case or not? Supposing it is the latter, if we choice different transformation donor according to breeding aim, could grain quality characters be other result? These are questions which need to be studied more.

Acknowledgments:

The study is Supported by National Science Foundation of China (No.10505018), the grant "Application of Nuclear Techniques in Agriculture" from the Chinese Ministry of Agriculture (No.200803034) and Henan province Zhengzhou science and technology bureau brainstorm project （No.083SGYS33261-10）.

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1/09/2011

Effect of Mercuric Oxide Toxicity on some Biochemical Parameters on African Cat Fish *Clarias gariepinus* Present in the River Nile

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Abstract: The effect of dietary carbohydrates and mercuric oxide on haematalogical profile, blood chemistry and hormonal level was studied in African cat fish (*Clarias gariepinus).* Fish were divided into 3 groups (n=10), exposed to different doses of mercuric oxide and carbohydrate. Group (1) was served as control. Group (2) was fed with carbohydrate and mercuric oxide (10 mg Kg^{-1} diet ration). Group (3) was fed with carbohydrate and mercuric oxide (1 5 rng Kg^{-1} diet ration). There is a significant decrease in hemoglobin and P .C.V in group (3). There is a significant increase in serum corlisol, cholesterol, AST, ALT, urea, creatinine and alkaline phosphorous in group (3). Also there is a significant decrease in serum phosphorous, sodium and potassium in treated fish. There is a significant high level of mercuric content in kidney muscles, heart and spleen in group (3) suggesting toxic effects of mercuric oxide on African cat fish (*Clarias gariepinus).* The total viable count of bacteria identified higher in fish fed on carbohydrate mercuric. Predominate bacteria were identified as, E. coli, Streptococcous, Pseudomonas, and Fluorscences. We emphasize the finding that an increase carbohydrate concentration causes harmful pathological effect which reduces humoral immure responses and enhances dietary mercuric toxicity.
[Mona, S. Zaki, Nabila, Elbattrawy Olfat M. Fawzi, Isis Awad and Nagwa, S. Atta. **Effect of Mercuric Oxide Toxicity on some Biochemical Parameters on African Cat Fish *Clarias gariepinus* Present in the River Nile.** Life Science Journal. 2011;8(1):363-368] (ISSN:1097–8135). http://www.lifesciencesite.com.

Keywords: *Clarias gariepinis*, mercuric pollution, haematalogical, biochemical, clinicopathological, Bacteria account

1. Introduction:

Fish plays an important role, not only in human food diets but also in animal and poultry rations. It is a palatable and easily digested food which is rich in vitamins, calcium, phosphorous and iodine. In Egyp, fish is considered as a cheap food article if compared with other foods of animal origin. The flesh of healthy fish is considered as a marker for the natural aquatic environment.

In animals, mercuric oxides cause inhibition of certain enzymes, which has several neurological effects. Next to the neurological effects vanadium can cause breathing disorders, paralyses and negative effects on the liver and kidneys. Laboratory tests with test animals have shown that mercuric and vanadium can cause harm to the reproductive system of male animals and rat it accumulates in the female placenta. Vanadium can be found in fishes and many other species. In mussels and crabs mercuric and vanadium strongly bioaccumulates, which can lead to concentrations of about 10^5 to 10^6 times greater than the concentrations that are found in seawater [1-8]

In recent years, much attention had been paid to the possible danger of metals poisoning in human as a result of consumption of contaminated fishes. So, the present study was carried out to elucidate the impact of mercuric oxide on African cat fish (*Clarias gariepinus).* It's haematological, biochemical and hormonal parameters were studied as well as the bacteriological and clinopathological investigation.

2. Material and methods

Experimental design:

Thirty African cat Clarias *gariepinis* were used to assess the effects of mercuric oxide. Fish weighting from 180-250 were obtained from Nile river and were kept in glass aquaria supplied with dechlorinate tap water at rate of one litter for each cm of fish's body. Fish were acclimated to the laboratory conditions for two weeks before the beginning of the experiment, they were fed a commercial fish diet [9], the composition of diet is illustrate in table (1), the experiment was determined after 4 weeks. Fish were divided into three groups (n=10) and exposed to different doses of mercuric oxide and carbohydrate. Group (1) was served as control, group (2) was fed with carbohydrate and mercuric oxide (10 mg kg-1 diet rations), group (3) was fed with carbohydrate and mercuric oxide (15 mg/kg^{-1} diet ration).

Mean of the initial body, weight of the each examined fish at the beginning of the experiment then after 2-4weeks of exposure were determined.

Blood samples:

Blood samples were collected from the caudal vein after 4 weeks of exposure. Each sample was divided into two parts the first one was heparinized for haematological investigations, while the second was centrifuged at 3000 rpm for 5 minutes to obtain serum for biochemical studies.

Hematological Analysis:

Haematological studies were performed according to Sandnes *et al.* [10], where blood haemologlobin (Hb) and haematocrit (Ht) values were evaluated.

Biochemical Analysis:

The activities of alkaline phosphatase, aspartic aminotransferase (AST) and alanine aminotrarsferase (ALT) as well as cholesterol urea and creatinine level were determined according to the method of Varley *et al*. [11] by using commercial kits (Bio Merieus, France)

Total scrum protein was estimated according to Drupt [12]. Serum cortisol was analyzed by a Gamma counter using 125 I cortisol radioimmunassay Kit) Baxter Health Care Corporation USA) according to the method described by Pickering and Pottinger [13]. Potassium, Sodium and Phosphorous concentrations were determined by atomic absorption spectrophotometer [11].

Tissue analysis:

Liver, kidney and spleen samples were washed with distilled water then dried in hot air oven. sulphuric acid and hydrogen peroxide were added on samples then heated until the mixture became transparent after performing a wet ash digestion according to the method of Issac and Kerber [14].

Identification ion of bacteria:

The liver, kidney, spleen, muscle, stomach and gill from each examined fish were diluted immediately after sampling in sterile 0.9% saline and 0.1 ml volumes of appropriate dilutions and were spread over the surface of the typtic soy agar (oxide).The plates were incubate at $22^{O}C$ and inspected daily for up to 4 weeks.

The isolates were classified and identified according to Steverson [15] and Quirm e*t al.* [16].

The data were evaluated statistically according to Gad-Weil [17].

Water samples:

Two water of samples were collected from River Nile (Hawamdya) as well as two water samples from any heavy metal pollution El-Kasr El-Eini (control) were analyzed for mercuric concentration by atomic absorption spectrophotometer.

3. Results and Discussion:

Data in Table (1) showed that, the mercuric oxide level in Hehvan region was clearly higher than the maximum allowable concentration for human consumption as recommended internationally according to WHO (World Health Organization). Nadal *et al*. [2] concluded that the occurrence of mercuric in nature and its use in various industrial processes has increased its inputs in the environment. From the present study it is clear that the low mercuric levels were reported in water samples collected from areas far from industrial discharges, while high mercuric levels in the present study may be due to the collection of samples from areas subjected to industrial pollution.

In Table (3) there is a significant decrease in body weight in group 3 (fish fed 15 mg/kg diet mercuric oxide for 4 weeks) than in group 1 (control) and group 2 (fish fed l0 mg mercuric), this results agree with that reported by Khalaf-Allah [18].

The results present in Table (5) showed the cholesterol levels between different groups. The level was significantly increased in group 3 (fish fed on l5 mg vanadium) than in group 1(control). Hypercirolestremia might be due to necrotic changes occurring in liver with liberation of cholesterol as a byproduct of cell destruction. The present data suggest that impaired liver function lead to increased serum levels of alkaline phosphat, AST and ALT among group 3 (fish fed on l5 mg mercuric) and among group 2 (fish fed 10 mg mercuric) compared with group 1(control). In this concern Khalaf-Allah [8] concluded that ALT and AST enzymes are good indices for the health status of liver parenchymatous, tissue necrosis is considered as the main source of AST and its increase in the serum of African cat fish *(Clarias Gariepinus)* and declared these necrotic changes [18]. In addition, exposure of fish to environmental pollutants might result in stimulation or depression of the enzyme activity depending on the concentration of pollutant and the duration of exposure [19, 20].

Regarding the effect of mercuric oxide on serum cortisol level in African cat fish (*Claias gariepinus)* highest level was obtained in group 3 (fish fed on 15 mg mercuric) then in group 2 (fish fed on 10 mg vanadium) as compared to that obtained in group 1 (control). The significant increase of

cortisol level is probably due to the activation of hypothalamus pituitary internal axis [21].

From the data present in Table (5), it is clear that elevation of mercuric oxide level in the diets fed to *(Clarias gariepinus)* was positively correlated to hemoglobin (Hb) levels and haematocrit (Ht). A marked decrease in the IIb and Ht was recorded after feeding diet containing 15 mg and 10 mg mercuric , respectively. Reduced Hb reflects metabolic adjustment according to reduced need for oxygen by change in blood PH.

Moyle and Ceeh, Hall and Cliffs recorded actived acetchlinesterase of erthrocytes [22, 23] Further more Pickeringand Dusten [24] concluded that a consistent effect of cortisol was the reduction in the hemoglobin and iron levels as a result of decrease in appetite in rainbow trout fish or more likely to be the direct-result of catabolic effect of cortisol in the fish tissues [24].

The mean phosphorus, sodium and potassium values in the serum of fish of group 3 (fish fed 15 mg mercuric oxide) were significantly increased respectively than those recorded in the group 1 (control). This retention maybe attributed to kidney dysfunction, whereas, the kidney is the normal pass for sodium and potassium.

Kidney dysfunction may also explain the increase in serurn urea and creatinine especially in group3, but little known about the mechanisms involved in this association.

The results displayed also in Table (5) showed that there was general decrease in the mean total protein value in serum samples collected from the fish of group 3 and 2, respectively. The mean value of these parameters was lower than in group 1. Jagadeesh *et al*. [25] estimated marked decrease in glycogen in tissues of fresh water fish after exposure to vanadium [25].

This experiment showed that the body weight of the examined fish was significantly decreased than the initial body weight after 4 weeks of exposure to 15mg mercuric oxide. Also, Hilton and Better [25] recorded a significantly reduced growth and increased mortality among feeding diets of mercuric (0, 10, 100, 1000, 10000mg Kg-') [26]. The increase in muscles and tissue lactic acid (2 fold) in association with decrease in pyruvic acid (72 in muscles +26% in liver) reflect a shift towards an anaerobic metabolism of fish following long term exposure to mercuric [26]

Table (6) showed that, the bacterial isolates and counts were increased by feeding the fish with CHO and mercuric. The carbohydrates affect immunity and resistance to infection as recorded by Waagbo et al. [19] Utility of vanadate, mimetic protein phosphate inhibitors to protect fish from microorganism [27]. The increase of bacterial count among the fish fed on mercuric may be related to the increased level of corlisol which decreases the host immunity.

In the course of experiment, a high concentration or mercuric levels has been found in kidney, liver, spleen, heart and muscles of cat African catfish *(Clarias gariepinus)* fed 15 mg mercuric (Table 4). This suggests that these organs could be useful as a marker for vanadium in the aquatic environment. In this concern Ray et al. recorded a high concentration of mercuric in kidney liver and other organs of African cat fish as the concentration of mercuric in the tissues increased with its concentration in the aquatic environment and exposure time[28].After exposure of fish to increased doses for 4 days, the mercuric content in the muscle then increased in all tissues [20, 25, 26] The capability of mercuric to be present in fish muscle is of particular interest in assessing the exposure of man to environmental mercuric as ingested by food.

Clinicopathological observations:

Abnormal swimming lighting of the skin, scale loss and haemorrhasges, water seen on the external body surface. In addition to congestion of gills, eyes mouth, liver, kidney, spleen, and intestine. This was notice in fish exposed to mercuric oxide 15mg (group 3) but not in fish exposed to mercuric oxide 10 mg (group 2).

In conclusion: we emphasize that, the reported finding increase of carbohydrate concentrations causes harmful physiological effects, reduces hormonal immune response and enhances dietary toxicity.

Table 1: Ingredients and Proximate composition of diets used in the experiments with mercuric oxide

Ingredients	Diet Control	Diet 2	Diet 3
Fish meal	25	25	30
Meat and bone meal	5	5	10
Wheat bran	20	20	20
Skimmed milk	12	12	7
Yeast	10	10	15
Starch	-	10	15
Cod liver oil	2	2	2
Vitamin premix	1	1	1
Mercuric oxide	-	10	15
Crude protein %	40.35	35.95	38.89
Metabolizable energy k cal/kg]	2205.4	2551.78	2315.4
Ether extract%	4.29	4.21	2.86
Crude fiber %	4.46	3.73	4.27
Ash%	5.56	6.26	10.25
Lysine%	2.13	1.88	2.29
Methionine %	0.62	0.55	0.613

Mineral and vitamin premix per/kg of pellet food
Vit A, 8000 g/u, vit D 900 g/u vit E/u, vit k 4mg, vit B2 3.6 niacin 20mg, pyridoxine 0.2mg Vit B1 25, Mn 70mg, Se 60mg

Table 2: Mercuric oxide concentration in water samples collected from two areas in Egypt.

Areas	SampeNo.	Concentration of mercuric p.p.m
Hawamdya	1	1.05
	2	1.28
Ak-Kasr El-Aini	3	0.156
	4	0.164

Table 3: Changes in body weight in African cat fish (*Clarias gariepinis*) fed on different levels of dietery carbohydrate s in addition to mercuric oxide.

Weight / Group	Group 1	Group 2	Group 3
Initial body weight (g)	70±0.15	85±0.17	94±0.20
After 2 weeks (g)	100±0.45	102±0.24	97±0.62
After 4 weeks (g)	155±0.26	124±0.64	95±0.60*

Table 4: The mean mercuric concentration in the organs of fish mg/g net weight

Groups	Muscles	Spleen	Heart	Kidney	Liver
Group1	0.24±0.14	0.52±0.83	0.64±0.49	3.15±0.72	2.11±0.69
Group2	0.43±0.25	0.51±0.71	0.70±0.41	4.00±0.83	3.11±0.70
Group3	0.55±26	0.82±0.41	0.83±0.24	6.74±0.74	6.13±0.05

Table 5: Some haematological, biochemical parameters in African cat fish *Clarias gariepinis* on different levels of dietery carbohydrates in addition to mercuric oxide

Parameters/Group	Group 1	Group 2	Group 3
Hemoglobin g/dl	36.20±0.26	36.2±0.28	31.5±0.25*
HCT %	0.82±0.22	0.95±0.11	1.40±0.68*
Cartisol ng/dl	9.6±0.62	92±0.26	81±0.77*
Phosphorous mg/dl	122±1.24	111±0.75	103±0.14*
Sodium M.EQ	6.23±0.82	6.02±0.44	61±0.74*
Potassium M. EQ	20.42 ±3.2	21±0.73	26±0.72*
Alkphosphatase U/L	135±0.41	134±0.88	140±0.23*
AST U/L	23±0.17	25±0.74	36±0.28*
Cholestrol mg	140±0.25	145±0.13	161±0.54*
Total protein g/dl	8.2±0.76	8.02±0.81	7.02±0.72*
Urea mg/dl	2.1±0.78	3.4±0.76	4.8±0.23*
Creatinine mg/dl	0.75±0.23	0.72±0.76	0.90±0.52*

Table 6: Bacterial isolates recorded from the examined fish

Groups	Bacterial isolates	Site of isolation	Bacterial count
Group 3 (n= 10)	-E. Coli	-Muscles	-----
	-Streptococcus	-External surface, Stomach	$2X10^3$
	-E. Coli	Gills	----
	-Aeromonas	Gills, Stomach	$6.6X10^4$
Group 2 (n= 10)	-Enterbacter	Liver, Kidney	$1X10^3$
	-Pseudomonas	-Spleen, Muscles	$4X10^3$
	-Fluroscences	-Stomach	$2X10^6$
	-Lactobacillus	-Gills	----

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12/11/2010

Preoperative Seminal Plasma Disturbed Oxidant/Antioxidant Milieu Could Predict Failure of Varicocelectomy as a Therapeutic Modality for Male Infertility

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Abstract: Objectives: To evaluate seminal plasma (SP) malonaldehyde (MDA), superoxide desmutase (SOD) and CoQ10 levels in infertile men with and without varicocele as a trial to evaluate the predictability of their preoperative estimation for postoperative (PO) improvement of seminal parameters. Patients & Methods: 70 infertile men; with (n=35) and without (n=35) and 20 fertile men with (n=10) and without (n=10) clinically and ultrasonographically diagnosed varicocele. Infertile men with varicocele were assigned to undergo bilateral varicocelectomy using subinguinal approach. All infertile patients had preoperative hormonal profile. Semen samples were obtained from all men for standard semen analysis and SP estimation of CoQ10 using high-performance liquid chromatography (HPLC), SP MDA using a thiobarbituric acid reactive substances (TBARS) assay and SP SOD activity by chemiluminescence. Another semen sample was obtained PO for standard semen analysis. Results: Baseline data showed a significant decrease of sperm concentration and percentage of sperms with rapid progressive motility with significantly higher percentage of sperms with abnormal morphology and significantly lower SP CoQ10 and SOD levels with significantly higher SP MDA levels in infertile men compared to fertile men with significant difference among infertile men in favor of those free of varicocele. In all infertile men, there was negative significant correlation between SP MDA and CoQ10 levels and a positive significant correlation between SP SOD and CoQ10 levels with higher significance in those had varicocele. In infertile men free of varicocele, there was a negative correlation between sperm count and SP MDA with a positive significant correlation with SP SOD levels, while in infertile men with varicocele, the correlation was significant between SP SOD and the three seminal parameters and between the percentage of abnormal sperm forms and SP MDA and CoQ10. PO sperm count showed a negative significant correlation with preoperative SP MDA levels, while the correlation was positive significant with SP CoQ10 and SP SOD levels. Regression analysis identified high preoperative SP SOD level as significant predictor of improvement of sperm count after varicocelectomy. ROC curve analysis defined preoperative SOD level at ≥88 U/ml as a specific predictor for PO improvement of sperm count, while identified preoperative SP MDA level at ≥0.53 nmol/ml and SP CoQ10 at cutoff point of 0.12 µg/ml could identify infertile men with varicocele most probably will not get benefit of varicocelectomy. Conclusion: Combined varicocele and disturbed oxidant/antioxidant system could be the underlying mechanism for varicocele associated male infertility and highly disturbed oxidant/antioxidant system could influence the outcome of varicocelectomy as a therapeutic modality. Preoperative estimation of SP levels of MDA and SOD could aid to predict the outcome of varicocelectomy.

[Ahmed M.Awadallah, Jehan H.Sabry and Mohamed M.El Sharkawy. **Preoperative Seminal Plasma Disturbed Oxidant/Antioxidant Milieu Could Predict Failure of Varicocelectomy as a Therapeutic Modality for Male Infertility.** Life Science Journal. 2011;8(1):369-376] (ISSN:1097–8135). http://www.lifesciencesite.com.

Keywords: Preoperative; Seminal Plasma; Oxidant/Antioxidant; Varicocelectomy; Infertility

1. Introduction:

Oxidative stress is believed to underlie the etiology of numerous human conditions. Organisms are subject to oxidative stress from endogenous and exogenous sources which can induce severe macromolecular, cellular and tissue damage through direct cytotoxic effects, promotion of primary genotoxic events, or generation of reactive oxygen intermediates such as the superoxide anion and hydroxyl radical, [(1)].

Reactive oxygen species (ROS) can be produced by human spermatozoa and as a result of high polyunsaturated fatty acid content, human spermatozoa plasma membranes are highly sensitive to ROS-induced damage and hydrogen peroxide appears to be the most toxic ROS for human spermatozoa. There is growing evidence that peroxidative damage to the human spermatozoa membrane is an important pathophysiological mechanism in human male infertility, [(2, 3)].

Human spermatozoa and seminal plasma possess various antioxidant systems to scavenge ROS and prevent ROS-related cellular damage. Failure of antioxidant defenses to detoxify excess ROS production can lead to significant oxidative damage including enzyme inactivation, protein degradation

and lipid peroxidation. Mitochondrial DNA is believed to be both source and target of ROS, and unlike nuclear DNA is not compactly packed and hence more susceptible to oxidative stress than nuclear DNA. Antioxidant defense systems, which are involved in a variety of detoxification reactions, exhibit baseline levels of activity to ensure the maintenance of the balance between production and removal of endogenous ROS and other pro-oxidants, [4, 5].

Varicocele is one of the leading causes of male infertility, and is present in almost 40% of infertile males. Understanding the role of oxidative stress in male reproduction has led some researchers to postulate oxidative stress as the possible cause of sperm dysfunction in varicocele patients, [6]. The objective of the present study was to evaluate the seminal plasma levels of MDA, SOD and CoQ10 as a sample of oxidant/antioxidant system in infertile men with and without varicocele and to conduct a trial to evaluate the predictability of their preoperative estimation for postoperative improvement of seminal parameters.

2. Patients and Methods:

This prospective selective comparative study was conducted at Clinical Pathology & General Surgery Departments, Benha University & Zagazig University Hospitals and was designed to comprise 35 infertile men with varied degrees of varicocele, 35 infertile men free of varicocele both clinically and by ultrasonographic examination and 20 age-matched fertile men; 10 had varicocele and 10 free of varicocele. All infertile patients underwent preoperative complete hormonal profile evaluation to exclude associated endocrinopathy.

All men were evaluated clinically in standing position with and without Valsalva maneuver for clinical detection and grading of varicocele, if present, as grade 1 (palpable with Valsalva), grade 2 (palpable without Valsalva), and grade 3 (visible through the scrotal skin). Ultrasonographic examination was conducted for assurance of varicocele grade, measurement of testicular dimensions, and testicular volume (in cm^3) was calculated by multiplying (0.53 length, width, depth). A testicular volume of <19 cm^3 was considered hypotrophic and a testicular volume difference between the right and left testicles of ≥3 cm^3 was considered asymmetric, [7, 8]. Infertile men with varicocele were assigned to undergo bilateral varicocelectomy using subinguinal approach.

Laboratory investigations
Sampling:

Semen samples were obtained from all men following a minimum of 3 days and a maximum of 5 days sexual abstinence. Immediately after sample donation, about 0.5 ml of the collected sample was obtained and stored in dark to protect against photodegradation of ubiquinone and frozen at -20°C till be assayed SP CoQ10 level. The remaining sample was allowed for liquefaction and divided into two parts: the first for a routine semen analysis, but the second part was centrifuged for 7 minutes and seminal plasma was removed and stored at -80°C till be assayed. Another semen samples were obtained PO from infertile men with varicocele for routine semen analysis.

Investigations:

Standard semen analysis, using light microscopy, was performed according to World Health Organization criteria [9] to determine sperm concentration, motility and morphology. Sperm motility was expressed as the percentage of sperms showed rapid progressive forward motility (those spermatozoa which exhibits actual space-gain motility). Morphological assessment was performed at x100 magnification under oil-immersion and results were expressed as the percentage of abnormal spermatozoa observed on each slide; normal spermatozoa is that has a smooth, oval configuration with a well-defined acrosome incorporating 40–70% of the sperm head, no neck, midpiece or tail defects, and no cytoplasmic droplets more than one-half the size of the sperm head.

CoQ10 was assayed by high-performance liquid chromatography (HPLC), employing a UV detector (275 nm), according to Littarru et al., [10]. Seminal plasma, 0.5 ml supplemented with 2 ml of ethanol:isopropanol (95:5), 0.5 ml of 0.1 M sodium dodecyl sulfate and 0.5 µg of coenzyme Q8 (CoQ8) as an internal standard, was extracted twice with 4 ml of n-hexane. Combined extracts were brought to dryness under nitrogen and re-dissolved in 100 µl of ethanol. An aliquot of 20 µl was injected into the HPLC apparatus, whose conditions were as follows: column, ultrasphere octodecylsilane, 250 x 4.6 mm; mobile phase, ethanol-methanol (70:30); detector, UV 275 nm. The levels were expressed as concentrations (µg/ml).

Malonaldehyde (MDA) measurements: lipid peroxide levels in the seminal plasma was measured using a thiobarbituric acid reactive substances (TBARS) assay, which monitors MDA production based on the method of Beuge & Aust [11]. Briefly, to 100 µl sample of seminal plasma, 200 µl of cold 1.15% (w/v) KCl was added to 1.8 ml of 3% phosphoric acid and 0.6 ml of 0.6% TBA. These mixtures were heated in boiling water for 45 min.

After cooling, the MDA was extracted by centrifugation at 1,500 × g for 10 min at 25ºC and the intensity was measured at 535 nm using ultraviolet-visible spectrophotometry. The MDA level was determined using the molar absorption coefficient of the MDA at 535 nm 1.56×105 mol/L/cm.

Superoxide dismutase activity was quantified by chemiluminescence [12] using the xanthine/XO lucigenin assay. A final volume of 1 ml contained the following components: 100 µl seminal plasma, 100 mM diethylenetriaminepentaacetic acid (DTPA); 100 mM lucigenin, 180nM XO, and 50 mM xanthine in 50 mM HEPES, pH 7.4. The reaction was started by adding xanthine, and the resulting photon emission was recorded in Bertold LB 9505 C luminometer at 25ºC. Bovine CuZn SOD was used for calibration. One unit represents the concentration of SOD required to inhibit the release of superoxide by 50% and equals 5 nM Cu.

Statistical analysis

Obtained data were presented as mean±SD, ranges, numbers and ratios. Results were analyzed using Wilcoxon Signed Ranks Test for comparisons between groups and Chi-square (X^2-test) for numbers and percentage comparisons. Possible relationships were investigated using Pearson's linear correlation coefficient and Regression analysis (Stepwise method) was used for identification of predictors for PO sperm count improvement. ROC curve analysis, judged by area under curve (AUC), was used for evaluation of cutoff points of each parameter as predictor. Statistical analysis was conducted using the SPSS (Version 10, 2002) for Windows statistical package. P value <0.05 was considered statistically significant.

3. Results:

There was non-significant (p>0.05) difference between studied groups as regards to age that ranged between 23.5 and 39.5; mean: 30.6±2.7 years. Infertile group comprised 70 patients had a mean duration of infertility of 3.6±1.4; range: 1-6 years with a non-significant (p>0.05) difference between both infertility subgroups. Twenty-three testicles in infertile group were hypotrophic, while only 4 testicles were hypotrophic in fertile group, with a significantly (X^2=4.356, p<0.01) higher frequency of hypotrophic testicles in the infertile patients and non-significantly higher frequency among testes free of varicocele compared to those had varicocele, (Table 1). Among infertile patients with varicocele, 15 patients had bilateral varicocele of grade III, 12 patients had bilateral varicocele of grade II and 8 patients had bilateral varicocele of grade I, while among fertile men with varicocele, 5 patients had bilateral varicocele of grade I, 3 patients had bilateral varicocele of grade II and only 2 patients had bilateral varicocele of grade III.

Baseline seminal parameters showed a significant (p<0.05) decrease of sperm concentration and percentage of sperms with rapid progressive motility with significantly higher percentage of sperms with abnormal morphology in infertile compared to fertile men and in those had varicocele compared to those free of varicocele. Varicocelectomy significantly improved sperm concentration and percentage of motile sperms and significantly reduced the percentage of sperms with abnormal forms (Table 2).

Baseline SP CoQ10 and SOD levels were significantly lower and SP MDA levels were significantly higher in infertile men compared to fertile men with significant difference between infertile men in favor of those free of varicocele (Table 3).

In all infertile men, there was negative significant correlation between SP MDA and CoQ10 levels and a positive significant correlation between SP SOD and CoQ10 levels with higher significance in those had varicocele. Moreover, there was a negative correlation between SP SOD and MDA in infertile men and was significant in those had varicocele (Table 4).

The impact of disturbed testicular oxidative milieu on sperm quality was manifested as negative significant correlation between sperm count in infertile men without varicocele and SP MDA with a positive significant correlation with SP SOD levels. Such impact was intensified by the presence of varicocele in infertile men with varicocele, where the correlation between SP SOD was significant with the three seminal parameters and correlation between the percentage of abnormal sperm forms and SP MDA and CoQ10 became significant (Table 4).

Moreover, in infertile patients with varicocele, PO sperm count showed a negative significant correlation with preoperative SP MDA levels, (r=-0.4, p=0.029), while the correlation was positive significant, (r=0.432 & 0.498, p=0.002, respectively) with SP CoQ10 and SP SOD levels (Table 4, Fig. 1).

Regression analysis of preoperatively estimated SP parameters identified high SP SOD level as significant predictor, in 2 models, of improvement of sperm count after varicocelectomy, while high preoperative SP MDA level was significant in only one model and its exclusion minimal affected significance of preoperative SP SOD as a predictor (Table 5).

For cutoff point verification, ROC curve analysis defined preoperative SOD level at ≥88 U/ml

as a specific predictor for postoperative improvement of sperm count with AUC=0.621, (Fig. 2), while preoperative SP MDA level at ≥0.53 nmol/ml could identify infertile men with varicocele most probably will not get benefit of varicocelectomy with AUC=0.823 (Fig. 3). On the other hand, preoperative SP CoQ10 level could be used as screening test with high sensitivity for the prediction of varicocelectomy failure at cutoff point of >0.12 µg/ml with AUC=0.270 (Fig. 4).

Table (1): Testicular dimension data

			Testicular size		Testicular symmetry	
			Mean±SD (cm^3)	Number of hypo-trophic testes	Testicular size difference (cm^3)	Number of asymmetric
Fertile men	Free of varicocele	Rt	26.3±2.4	1	2.43±0.6	4
		Lt	23.8±2.8	2		
	varicocele	Rt	23.7±2.1	0	2.44±1	1
		Lt	21.2±2.2*†	1		
Infertile men	Free of varicocele	Rt	21.6±4.9†	6	2.12±0.9	4
		Lt	21.1±5.5†	9		
	varicocele	Rt	23±2	3	2.45±1.17	5
		Lt	20.9±2.1*†	5		

Data are presented as means±SD & numbers
Hypotrophic testis means testicular size <19 cm^3
Asymmetric testes means testicular size difference >3 cm^3
*: significant versus right testicular size.
†: significant versus fertile men free of varicocele.

Table (2): Seminal analysis data recorded in studied groups

Group / Parameter	Fertile Men		Infertile Men		
	Free of varicocele	With varicocele	Free of varicocele	With varicocele	
				Preoperative	Postoperative
Count (10^6/ml)	67.1±13.6	39.9±15.7	28.3±7.5	20.5±5.3	24.1±6.3
	Z=2.805, p_1=0.005		Z=5.627, p_1<0.001		Z=5.221, p_4<0.001
			Z=7.156, p_2<0.001	Z=4.763, p_3<0.001	
Motility (%)	72.9±11.3	64±8.2	48.7±8.5	34.1±5.9	54.1±9.4
	Z=1.788, p_1>0.05		Z=5.328, p_1<0.001		Z=5.163, p_4<0.001
			Z=6.354, p_2<0.001	Z=4.154, p_3<0.001	
Abnormal forms (%)	13.8±4.2	22.2±4.3	25.9±8.1	34.2±10.6	23.9±7.4
	Z=7.156, p_1<0.001		Z=4.763, p_1<0.001		Z=5.162, p_4<0.001
			Z=7.156, p_2<0.001	Z=4.763, p_3<0.001	

p_1: significance versus men free of varicocele irrespective of fertility
p_2: significance versus fertile men with varicocele p_3: significance versus fertile men free of varicocele
p_4: significance versus preoperative measures

Table (3): Baseline SP MDA, CoQ10 and SOD levels estimated in studied groups

	Fertile		Infertile	
	With varicocele	Free of varicocele	With varicocele	Free of varicocele
MDA (nmol/ml)	0.34±0.03	0.45±0.046	0.54±0.08	0.48±0.07
Statistical analysis	Z=2.713, p_1=0.007		Z=2.807, p_1=0.005	
			Z=3.156, p_2=0.001	Z=1.432, p_3>0.05
CoQ10 (µg/ml)	0.25±0.032	0.2±0.043	0.11±0.031	0.16±0.035
Statistical analysis	Z=2.397, p_1=0.017		Z=4.624, p_1<0.001	
			Z=5.392, p_2<0.001	Z=2.245, p_3=0.025
SOD (U/ml)	106.3±11	121.3±8.1	87.4±10	96.5±8.4
Statistical analysis	Z=2.346, p_1=0.019		Z=3.027, p_1=0.002	
			Z=7.576, p_2<0.001	Z=8.468, p_3<0.001

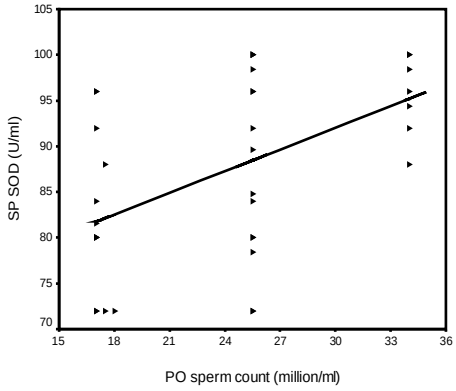
p_1: significance versus men free of varicocele irrespective of fertility
p_2: significance versus fertile men with varicocele p_3: significance versus fertile men free of varicocele.

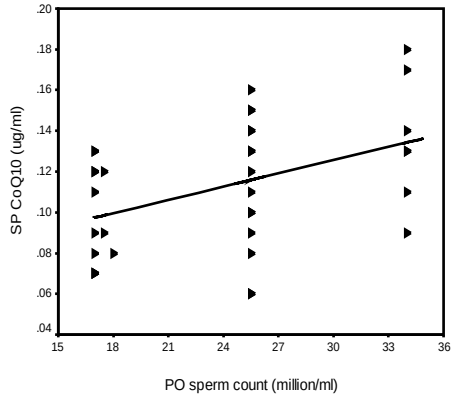
Table (4): Correlation coefficient between estimated seminal parameters in infertile men and with PO sperm count in infertile patients with varicocele

	Infertile with varicocele						Infertile free of Varicocele					
	SP MDA		SP CoQ10		SP SOD		SP MDA		SP CoQ10		SP SOD	
	r	p	r	p	r	p	r	p	R	p	r	p
Sperm count	-0.14	>0.05	0.312	>0.05	0.541	=0.001	-0.44	0.008	0.238	>0.05	0.489	=0.003
Sperm motility	-0.15	>0.05	0.283	>0.05	0.444	=0.007	-0.13	>0.05	0.254	>0.05	0.169	>0.05
Abnormal forms	0.393	=0.035	-0.512	=0.002	-0.42	=0.011	0.234	>0.05	-0.11	>0.05	-0.3	>0.05
SP MDA			-0.39	=0.022	-0.54	=0.001			-0.37	=0.042	-0.16	>0.05
SP CoQ10					0.523	=0.001					0.44	=0.008
PO sperm count	-0.4	=0.029	0.432	=0.002	0.498	=0.002						

Table (5): Regression analysis of estimated seminal plasma parameters for the predictability of PO improvement

Model	Parameter	Standardized coefficient	t	Significance
Model 1	SP SOD	0.498	3.302	=0.002
Model 2	SP SOD	0.710	4.210	<0.001
	SP MDA	0.391	2.318	=0.027





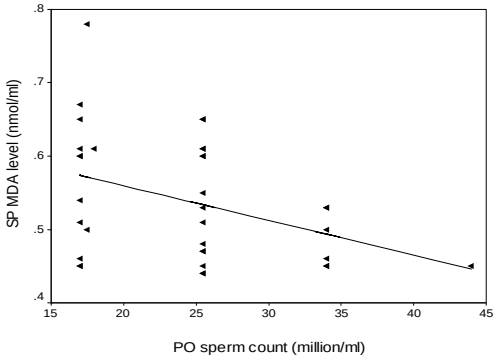


Fig. (1): Correlation between PO sperm count and preoperatively estimated SP SOD, CoQ10 and MDA levels in infertile patients with varicocele.

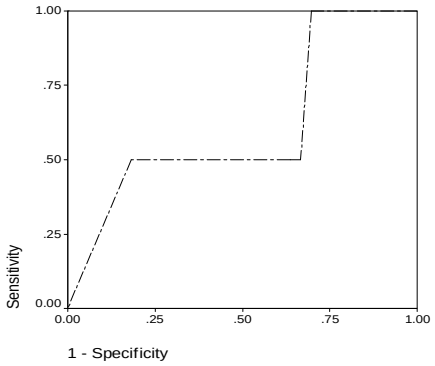


Fig. (2): ROC curve analysis of preoperative seminal plasma SOD level at cutoff point of ≥88 U/ml as a predictor for postoperative improved seminal characters

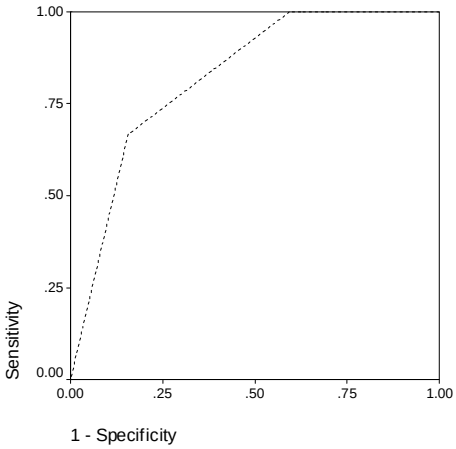


Fig. (3): ROC curve analysis of preoperative seminal plasma MDA level at cutoff point of ≤0.53 ng/ml as a predictor for postoperative improved sperm count

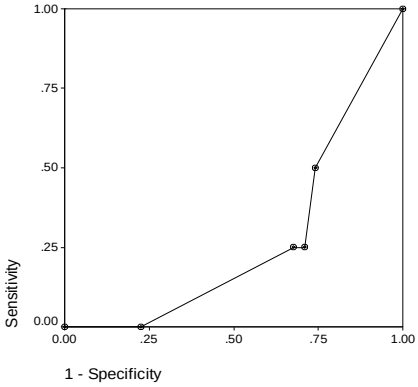


Fig. (4): ROC curve analysis of preoperative seminal plasma CoQ10 level at cutoff point of 0.12 µg/ml as a predictor for postoperative improved sperm count

4. Discussion:

The current study reported significant improvement of seminal parameters after varicocelectomy using the subinguinal approach with special regard to the percentage of progressive forward motile sperms. These data point to the beneficial effect of varicocelectomy on the fertility status of infertile couple due to a male factor and go in hand with Ficarra et al. [13] who found varicocele repair has a beneficial effect on fertility status in infertile men with palpable varicocele with a significant increase in pregnancy rate in patients who underwent varicocele treatment (36.4%) compared with patients having no treatment (20%). Moreover, French et al. [14] reported that with regard to the efficacy of varicocele repair, previous meta-analysis of the available data has been misleading due to improper selection criteria and the findings of the Cochrane database review, a study that has been accepted by many as evidence against varicocele repair; however, varicocele repair was found not only as an effective treatment for appropriately selected patients but can also be the most cost effective option.

The impact of disturbed oxidant/antioxidant milieu on testicular function and spermatic capacitation was evident and manifested as higher levels of SP MDA and lower SP CoQ10 and SOD in infertile men free of varicocele compared to fertile men free of varicocele. These data go in hand with multiple studies previously evaluated the impact of disturbed oxidant milieu on testicular functions; Aydemir et al. [15] suggested that Cu and Fe might be mediators of the effects of oxidative damage on spermatogenesis and male infertility and the determination of Fe and Cu levels in serum and seminal plasma during infertility investigation is recommended. Li et al. [16] reported significant difference in SP CoQ10 concentrations between fertile and infertile men. Aydemir et al. [17] suggested that the susceptibility of sperm and seminal plasma to oxidative stress is significantly greater in idiopathic infertile men with the glutathione S-transferase Mu-1 null genotype compared with those possessing the gene and therefore, in patients with idiopathic infertility, GSTM1 polymorphism might be an important source of variation in susceptibility of spermatozoa to oxidative damage.

The risk of oxidative damage was intensified by the presence of varicocele as manifested by significantly higher SP MDA with lower SP SOD and CoQ10 in infertile men had varicocele compared to those free of varicocele. In support of this finding, there was a positive significant correlation between seminal parameters and SP SOD and CoQ10 and negative with SP MDA; a finding indicating the deleterious effect of presence of varicocele on the disturbed oxidant/antioxidant milieu with its harmful effect on testicular function. Also, the reported positive significant correlation between PO improvement of sperm count and preoperative SP SOD and negative significant correlation with SP MDA level indicated the effect-outcome relationship between preoperative antioxidant capacity and on coming improvement.

These data were further supported statistically where preoperative SP SOD and MDA levels were found as predictor for postoperative change of sperm count and their high levels are specific predictors for outcome, improvement or failure, respectively. These data go in hand with Giulini et al. [18] who evaluated total antioxidant capacity in the seminal plasma of infertile patients with varicocele in relation to their semen parameters

and found seminal plasma total antioxidant capacity concentrations were significantly lower than in controls and normozoospermic patients with varicocele and in patients with severe oligosthenozoospermia than in asthenozoozpermic patients with varicocele and in all subjects, concentrations of TAC showed a positive correlation with sperm concentration and motility.

Also, Abd-Elmoaty et al. [19] reported significantly higher levels of oxidants (malonaldehyde and nitric oxide) and reduced levels of antioxidants (superoxide dismutase, glutathione peroxidase, catalase, and ascorbic acid) are seen in semen of infertile men with varicocele and seminal oxidative stress seen in men with varicocele is associated with sperm motility and grade of varicocele.

In hand with the applicability of estimation of seminal plasma prior to initiation of treatment could predict the treatment outcome, Vankatesh et al. [20], (2009) recommended measurement of seminal reactive oxygen species levels in infertile men for better understanding of the aetiology and selection of antioxidant regimen in the treatment of male infertility. Moreover, Ozbek et al. [21] found a statistically significant difference between mean preoperative and postoperative seminal NO levels, whereas there was no significant difference between mean postoperative seminal NO levels and that of control group and concluded that preoperative increased level of seminal NO levels may play a role in the sperm dysfunction in infertile patients with varicocele and its persistent level may explain failure to achieve fertility.

However, the current study found the highest diagnostic yield for improvement of sperm count will be associated with SP SOD level ≥88 U/ml and SP MDA level ≤0.53 nmol/ml, to our knowledge this is the first study tried to define cutoff points for these SP parameters for defining the surgical outcome of varicocelectomy

In conclusion, combined varicocele and disturbed oxidant/antioxidant system could be the underlying mechanism for varicocele associated male infertility and highly disturbed oxidant/antioxidant system could influence the outcome of varicocelectomy as a therapeutic modality. Moreover, preoperative estimation of SP levels of MDA and SOD could aid to predict the outcome of varicocelectomy. However, proposed cutoff points need further larger scale studies for confirmation.

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12/22/2010

Evaluation of *Colletotrichum graminicola* as an Eventual Bioherbicide for Biocontrolling *Alisma plantago-aquatica* in Paddy Fields

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Abstract: *Alisma plantago-aquatica* is one of the most important paddy field weeds in Iran. In this research, *Colletotrichum graminicola* was isolated from the said weed and studied as a biological agent for controlling *Alisma plantago-aquatica*. To do so, at first, reactions of five rice cultivars including three indigenous cultivars such as Hashemi, Ali Kazemi and Binam and two bred ones, i.e. Sepidroud and Khazar to *Colletotrichum graminicola* were evaluated. Thus, a random completely design with three replications and five treatments was used at a greenhouse. Then, *Colletotrichum graminicola* was inoculated on *Alisma plantago-aquatica*. This inoculation was done at the 3-4-leaf stage using a spore suspension consisting of 10^6 conidia/ml distilled water to which Tween-20 1% was added. Results indicated that the disease rating caused by the this fungus in the weed was more than that in the studied rice cultivars. Also, the fungus had a significant effect on the height, fresh weight and dry weight of *Alisma plantago-aquatica* and reduced them. Furthermore, *Colletotrichum graminicola* had a significant effect on all the studied rice cultivars and significantly reduced their heights and fresh weights. With consideration of the results of this research, *Colletotrichum graminicola* is not recommended as a probable mycoherbicide for biological controlling *Alisma plantago-aquatica* in paddy fields, unless more tolerant rice cultivars are used when using it.
[Mohammad Reza Safari Motlagh and Armin Javadzadeh. Evaluation of *Colletotrichum graminicola* as an Eventual Bioherbicide for Biocontrolling *Alisma plantago-aquatica* in Paddy Fields. Life Science Journal. 2011;8(1):377-382 (ISSN:1097–8135). http://www.lifesciencesite.com.

Keywords: *Alisma plantago-aquatica*; biological control; *Colletotrichum graminicola*; rice

1. Introduction

Usually, using chemical herbicides is the most effective method for controlling various weeds (Fryer and Chancellor, 1970). However, there are two obvious difficulties regarding the outcomes of using these herbicides which include extensive increase of tolerance to herbicides in weed populations and complexity of the eradication of weed races or crop families; that is, wherever herbicides are used extensively, interspecies selection is its most dominant result which depending on the type of chemicals that are used, creates many changes in the relative frequency of the species (Fryer and Chancellor, 1970; Harris et al., 2003). On the other hand, it was found that weeds tolerant to an herbicide may be tolerant to other herbicides as well (Kouchaki et al., 2001).

Recent biotechnology developments have increased scientists' interest in microorganisms as potential resources of natural herbicides (Kouchaki et al., 2001). In fact, the management of pathogens and insects is the basis of the biological control of weeds (Hoy and Herzog, 1985). Moreover, using plant pathogens, due to their specificity (specific performances) in terms of hosts and also their lower costing production compared with insects (except for obligate parasites) is very useful and cost-effective (Kouchaki et al., 2001). There are several examples of using different microorganisms such as fungi for the biological control of weeds, but their numbers in terms of controlling paddy field weeds is limited. For example, *Colletotrichum gloeosporioides* f.sp. *aeschynomene* is used as a microbial herbicide known as Collego for controlling important weeds in soybean and rice fields, i.e. *Aeschynomene virginica* (Bowers, 1986; TeBeest and Templeton, 1985).

Studies showed that of the pathogen fungi isolated from weeds such as *Sesbania exaltata, Solanum viarum* and *Striga hermonothica*, being considered as the main problems of soybean and rice cultivation, *Colletotrichum truncata* and *Ralstonia solanacearum* caused the highest disease ratings in the said weeds (Charudattan, 2001). In a study conducted in Vietnam, Australia and South Korea for biological controlling *Echinochloa crus-galli* and *Echinochloa* sp. in paddy fields, *Exserohilum fusiforme* and *Colletotrichum graminicola* were used and they prevented their propagation during the growth stages of rice (Johanson et al., 2003). Usually, *Alisma plantago-aquatica* is

considered as one of the most important rice weeds in Australia and Asia (Ash et al., 2005). This weed which is among broad-leaf and monocotyledon weeds, due to having too much seeds with long dormancy and its high resistance to many chemical herbicides has greatly propagated in paddy fields (Rezvani et al., 2002).

Investigations revealed that *Plectosporium tabacinum* and *Rhynchosporium alismatic* had considerable effects on controlling *Alisma plantago-aquatica* (Ash et al., 2005). Another study showed that *Alternaria eichhorniae* isolated from Water hyacinth only affected this weed and did not control *Alisma plantago-aquatica* (Martinez and Charudattan, 1998).

Usually, fungi such as *Fusarium oxysporum* and *Colletotrichum coccodes* being among good mycoherbicides are changed by pectinase, cellulase and expancine coding genes (enzymes which facilitate the influence and growth of fungi inside weed tissues) (Hershkovitz et al., 2007). Moreover, isolates from *Colletotrichum graminicola* have been used for the biological control of barnyardgrass (Yang et al., 2000).

In another study, *Colletotrichum dematium* was introduced for controlling *Epilobium angutifolium* which can replace herbicides such as simazine, atrazine (Watson and Winder, 1993).

Also, *Ipomoea lacunosa* is a dangerous and dispersed weed in soybean and cotton fields and many other plants all through the southern regions of the United States and the use of *Colletotrichum capsici* has proved effective in reducing its damages (Cartwright and Tempelton, 1994). Furthermore, a combination of glyphosate and *Colletotrichum truncatum* is effective for controlling *Sesbania exaltata* in a way that by using the said fungus, the level of the used herbicide decreases (Boyette et al., 2008). However, one of the prerequisites which should be observed is that prior to the introduction of each biological factor, ensuring that no probable damages would be made to crops is important. Hence, studying and investigating the reactions of some crop varieties which have been planted in places where a bioherbicide is used is necessary (Bergson and Carter, 2002). One of the most important principles of developing biocontrol is the existence of biological factor-resistant crops (Burdon and Leather, 1990).

The main objective of the present research was to evaluate *Colletotrichum graminicola* as a mycoherbicide for controlling *Alisma plantago-aquatica.* In order to do so, at first, the above-mentioned fungus was isolated from *Alisma plantago-aquatica* and then, its disease rating in the weed and some important rice cultivars of Guilan province in Iran was studied.

2. Materials and Methods

2.1 Collection and culture of fungal isolates

Leaves with symptoms of the disease *Alisma plantago-aquatica* were collected in Guilan province of Iran, cut to appropriate sizes and transferred to the laboratory. Samples were surface sterilized with 0.5% sodium hypochlorite solution, washed by sterile distilled water and placed on potato dextrose agar in petri dishes.Then, petri dishes were incubated at 28^0C in darkness or light on a 12 hours light/dark photoperiod for 6-15 days. Conidia were single-sporulated and then, monoconidial isolates of the recovered fungi were maintained on half-strength PDA slants in test tubes as stock cultures (Zhang et al., 1996).

2.2 Study and identification of fungi

Fungi which had grown were isolated and koch's postulates were completed for most sample after each collection. Cultures of these fungi were submitted to the Research Plant Pathology Institute of Iran for the confirmation of identification.

2.3 Pathogenicity test

This reaction occurred as complete random design (CRD) with one treatment and 3 replications. Inoculation of *Alisma plantago-aquatica* was performed at its 3-4 leaf stage in greenhouse. To do so, a spore suspension including 10^6 *Colletotrichum graminicola* spore/ml distilled water was used. In order to increase adsorption, 1% Tween-20 was used. Weeds were planted in plastic pots 2.5 cm in diameter containing farm soil,. For each treatment, one control was assigned (Zhang et al., 1996). Pots were placed at 25-30^0C, 12 D: 12 L photoperiod and a relative humidity of more than 90%,. This suspension was sprayed on the leaves using a sprayer. It should be mentioned that before inoculation, all pots were sprayed with distilled water. To create a relative humidity higher than 90%, treated plants were immediately covered with plastic bags for 48 hours (Ghorbani et al., 2000). Evaluation was done 7 days after inoculation based on lesion type and size in reaction to inoculation: 0= lesions absent, 1= small, unexpanded lesions, 2= slightly to moderately expanded lesions, 3= large lesions (Zhang et al., 1996).Then, five rice cultivars including 3 indigenous (Hashemi, Ali Kazemi and Binam) and 2 bred cultivars (Khazar and Sepidroud) were evaluated in complete random design with three replications against inoculation with *Colletotrichum graminicola*. In order to do so, first, rice seeds germinated and after being transferred to the greenhouse inside pots, 2.5 cm in diameter without any drain, they were planted in the farm soil. When the plants reached their 3-4 leaf stage, thinning was performed. Finally, there were 4 shrubs in each pot. Then, 2g urea fertilizer was added to the pots.

At this stage, inoculation was done by a spore suspension of *Colletotrichum graminicola* containing 10^6 spore/ ml of distilled water with 1% Tween-20. Other environmental conditions were similar to those of the weed. Evaluation was done 7 days after inoculation for which Horsfall-Barrat system was used. Then, disease ratings were calculated (Bertrand and Gottwald, 1997). It is noteworthy that in both experiments, one control was considered for each replication.

2.4 Measuring plant fresh weight, dry weight and height

In order to measure these traits, inoculated weeds and rice cultivars along with controls were transferred from greenhouse to the laboratory. Then, shrubs were cut on the soil surface and weighed by an electric scale. This weight was recorded as their fresh weight. After separately measuring their height, each shrub was placed inside a paper bag and for 48 hours, they were in an oven at 80-90^{0}C. When the bags were taken out of the oven, each shrub was weighed, which was considered as its dry weight (Ghorbani et al., 2000).

2.5 Data Analysis

Data analysis was done using SPSS and MSTAT-C softwares. In order to compare average values, Duncan test was used, while for comparing the reaction of rice cultivars, the difference between the average value of each fungus-treated rice cultivars and the controls and for weeds Chi-square test was used.

3. Results

According to the variance analysis table for the evaluation of the disease rating, it was found that the studied rice cultivars showed significant reactions to *Colletotrichum graminicola* (Table 1). Also, based on the comparison of the mean traits in the study of disease rating, the greatest effect of the fungus was seen on Sepidroud, i.e. this cultivar was less tolerant compared with others (Figure 1). Among indigenous cultivars, Hashemi showed less tolerance (Figure 1). There was no significant difference between Ali Kazemi, Khazar and Binam with only Binam being more tolerant to this fungus in terms of the number and sizes of the spots created (based on Horsfall-Barratt system) (Figure 1).

On the other hand, based on the variance analysis table for the evaluation of traits including height, fresh weight and dry weight, the studied rice cultivars showed significant reactions (Table 1). According to the comparison of the above-mentioned traits among the cultivars, it was found that for height, there was no significant difference between Hashemi, Sepidroud and Binam cultivars. Also, no significant difference was observed between Ali Kazemi and Khazar. No significant difference was found between the dry and fresh weights of Khazar and Binam as well (Table 2). However, a significant difference was observed in terms of these two traits between Hashemi, Ali Kazemi and Sepidroud (Table 2). Moreover, compared with other rice cultivars, Khazar showed more reductions of the said three traits (Table 2).

In the investigation of the reactions of the studied rice cultivars regarding their heights, fresh weights and dry weights compared with the controls, results showed that for height, Ali Kazemi, Sepidroud and Khazar were more affected by the fungus than the controls (in comparison with the controls, they revealed a decrease in height which compared with that of other cultivars was greater.); however, when compared with each other, they had no significant differences in terms of this trait (Table 3). In the second group, there were Hashemi and Binam for height decrease, yet with no significant difference between each other. But when compared with Ali Kazemi, Sepidroud and Khazar, they were less affected by the fungus (Table 3).

In terms of fresh weight, the said fungus was effective on Sepidroud (as a bred cultivar) and indigenous Hashemi and Ali Kazemi compared with the controls, but they showed no significant differences between themselves. *Colletotrichum graminicola* had no effects on the fresh weights of Khazar and Binam. Furthermore, these cultivars did not show any significant differences between themselves (Table 3). In comparison with the effect of the fungus on the fresh weight in bred cultivars, Sepidroud had more fresh weight decrease than Khazar.

Concerning dry weight, it was found that the fungus caused this trait to decrease in the studied cultivars compared with controls and that there was no significant difference between the cultivars.

And in terms of the effect of the said fungus on all the three studied traits, it was revealed that the fungus was more effective on height and fresh weight than on the dry weight. Moreover, it was found that bred cultivars were more sensitive to the fungus (Table 3).

Results from the present research showed that the disease rating caused by *Colletotrichum graminicola* in *Alisma plantago-aquatica* was more than that in the rice cultivars (Figure 1).

On the other hand, based on the Chi-square test, the above-mentioned fungus had a significant effect on all the three studied traits, i.e. fresh weight, dry weight and height (Table 4) and this effect was greater on height than on the other two traits (Table 4).

Table 1. Variance analysis of disease rating and the studied traits in rice cultivars affected by *C. graminicola*.

SOV	DF	Squares Mean			
		Disease rating	Height	Fresh weight	Dry weight
Treatment	4	0.778 **	171.278 **	13.814 **	0.834 **
Error	10	0.136	8.761	0.049	0.015
C.V.	-	15.62	4.21	4.49	14.19

** Significance at the probability level of 1%.
SOV: sources of variations
DF: degree of freedom

Table 2. Comparison of the reactions of rice cultivars affected by *C. graminicola.*

Cultivars	Height	Fresh weight	Dry weight
Hashemi	68.206 ± 0.586b	6.09 ± 0.0832b	1.013 ± 0.0166b
Ali Kazemi	82.833 ± 1.083a	7.956 ± 0.0633a	1.673 ± 0.155a
Sepidroud	69.206 ± 1.149b	4.683 ± 0.0392c	0.825 ± 0.0173b
Khazar	62.33 ± 2.385c	3.157 ± 0.0179d	0.407 ± 0.0121c
Binam	68.820 ± 2.454b	2.760 ± 0.261d	0.392 ± 0.0416c

Treatments having at least one similar letter do not show a significant difference at the probability level of 5%.

Table 3. Comparison of the reactions of rice cultivars affected by *C. graminicola* with those of the controls.

Cultivars	Change of Height	Change of Fresh weight	Change of Dry weight
Hashemi	-0.823 ± 0.151b	-0.036 ± 0.149a	-0.133 ± 0.33a
Ali Kazemi	-1.96 ± 0.571a	-0.046 ± 0.129a	-0.24 ± 0.14a
Sepidroud	-1.816 ± 0.183a	-0.063 ± 0.044a	-0.132 ± 0.048a
Khazar	-1.19 ± 0.052a	0.39 ± 0.084b	-0.121 ± 0.05a
Binam	-0.783 ± 0.859b	0.196 ± 0.066b	-0.118 ± 0.046a

Treatments having at least one similar letter do not show a significant difference at the probability level of 5%.

Table 4. Chi-square values of the studied traits affect by *C. graminicola* in weed.

Weed	Height	Fresh weight	Dry weight
Alisma plantago-aquatica	2.251 **	1.21 **	1.02 **

** : Significance at the probability level of 1%.

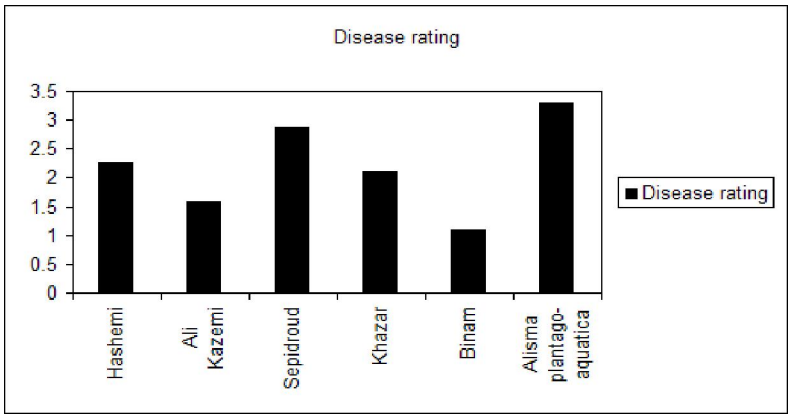

Figure 1. Diagram of the comparison of *Colletotrichum graminicola* mean disease rating in rice cultivars and *Alisma plantago-aquatica.*

4. Discussion

With consideration of the studies conducted in this research, *Colletotrichum graminicola* had a negative effect on both indigenous and bred studied rice cultivars. However, there were some differences between these two groups of cultivars in terms of their tolerance. As has been shown in other studies, different species of *Colletotrichum* including *C. graminicola* and *C. truncata* are considered as effective antagonistic fungi for controlling barnyardgrass and woody nightshade, but in order to extend their uses, the existence of tolerant cultivars is necessary for wheat and rice cultivation (Johanson et al., 2003).

Studies showed that *Colletotrichum graminicola* was effective on *Sorghum halepenes* (one of the weeds in alfalfa fields), but it also caused disease in alfalfa and this prevented the introduction of the said fungus as a biological agent (Templeton and Henry, 1990). Other studies on the reactions of bred and indigenous corn cultivars to *Colletotrichum* sp., isolated from annual mercury in corn fields in the US showed that the bred cultivars were more tolerant of disease rating, but the fungus was more effective on their dry weights yet with the existence of high disease rating in indigenous cultivars, the decrease of dry weight was not noticeable (17). Molecular studies showed that bred cultivars had more resistant genes, but in indigenous ones dry and fresh weight-controlling genes were even more (Norris, 1992). Also, studies showed that *Colletotrichum truncata* and *Ralstonia solanacearum*, which were effective for the biological control of *Sesbania exaltata* and *Striga hermonthica* and caused higher disease ratings, had fewer effects on soybean and rice bred cultivars while causing the same severe symptoms in indigenous ones (Charudattan, 2001).

Therefore, with consideration of the results from this research and similar studies it was concluded that though the disease rating caused by *Colletotrichum graminicola* in *Alisma plantago-aquatica* was more than in the studied rice cultivars, but with the negative effect of the fungus on the experimented traits taken into account, this fungus could not be introduced as a biological agent for controlling *Alisma plantago-aquatica* unless upon using it as a bioherbicide, other rice cultivars are cultivated which are more tolerant to *Colletotrichum graminicola* and this would require conducting more studies and also modifying rice cultivars by doing biotechnological and genetic engineering researches.

Acknowledgments:

This research was supported by the Islamic Azad University, Rasht Branch, Iran.

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10/1/2011

Investigation of the possibility to prepare supervised classification map of gully erosion

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Abstract: This study was done to investigate the possibility providing gully erosion map by the supervised classification of satellite images (ETM+) in two mountainous and plain land types. These land types were the part of Varamin plain, Tehran province, and Roodbar sub-basin, Guilan province, as plain and mountainous land types, respectively. The position of 652 and 124 ground control points were recorded by GPS respectively in mountain and plain land types. Soil gully erosion, land uses or plant covers were investigated in these points. Regarding ground control points and auxiliary points, training points of gully erosion and other surface features were introduced to software (Ilwis 3.3 Academic). The supervised classified map of gully erosion was prepared by maximum likelihood method and then, overall accuracy of gully erosion map was computed. Results showed that the possibility supervised classification of gully erosion isn't possible, although it need more studies for results generalization to other mountainous regions. Also, when land uses and other surface features to increase in plain physiography, it will be decreased the classification of accuracy.
[Ali Mohammadi Torkashvand, Vahid Shadparvar. Investigation of the possibility to prepare supervised classification map of gully erosion. Life Science Journal. 2011;8(1):383-386] (ISSN:1097–8135). http://www.lifesciencesite.com.

Keywords: Supervised classification, Gully erosion, Map.

1. Introduction

Gully erosion causes damages to vulnerable agricultural lands, water pollution by soil particles and chemicals, and mudflows which may affect urban areas (Poesen & Hooke, 1977). In contrast to the effort during the last decades to investigate sheet (inter-rill) and rill soil erosion processes, relatively few studies have been focused on quantifying and/or predicting gully erosion (Martinez Casasnovas, 2003). Gullies can develop as enlarged rills, but their genesis is generally more complex, involving sub-surface flows and sidewall processes (Bocco, 1991). The geographic distribution of gullies is one of the most important information required for soil conservation. Large gully systems have received much attention from researchers using modern geospatial analysis (Mohammadi-Torkashvand, 2008; Zinck *et al.*, 2001; Martinez Casasnovas 2003; Martinez Casasnovas *et al.*, 2004).

The possibility to use the aerial photographs for soil mapping has been known for a long time (Goosen, 1967). Commonly they were used to support conventional geomorphological methods (Stromquist, 1990), and also for direct identification of sheet, rill and gully erosion (Frazier *et al.* 1983; Stromquist *et al.*, 1985). But it should be regarded that field survey and photo interpretation for gully erosion mapping at the national scale is time consuming and expensive (Raoofi *et al.*, 2004). The extension of the use of modern spatial information technologies, such as geographical information systems (GIS), digital elevation modeling (DEM) and remote sensing, have created new possibilities for research as a key for gully erosion mapping (Martinez Casasnovas, 2003) that is economical due to low costs as well as quickness (Raoofi *et al.*, 2004).

Gully erosion is a serious problem in many parts of the world, and particularly in Iran, because of climate, lithology, soils, relief and land use/cover characteristics. In Isfahan province as a pilot design, Rahnama (2003) investigated the possibility of preparation of soil erosion features map by aerial photograph interpretation and concluded similar results. He recommended satellite imagery and GIS for this aim. Sirvio *et al.* (2004) have investigated gully erosion hazard assessments in Taita Hills, SE-Kenya, applying airborne digital camera orthomosaics and GIS for small-scale studies and field measurements for large-scale studies. Detection of distribution and intensity of gully erosion and main factors affecting gully erosion were investigated within Taita Hills and changes during the last 50 years.

Raoofi *et al.* (2004) categorized rill and gully erosions in Taleghan basin-Tehran province by using visual interpretation of images derived from the fusion of ETM+ bands and Cosmos image. Also a map of ground truth from eroded regions was provided by using Cosmos image as well as visual interpretation and field observations. Measurements had indicated an approximate 80 percent accuracy for

the categorization. Allan James *et al.* (2007) investigated the ability of the ALS (Airborne Laser-Scanning) topographic data to identify headwater channels and gullies for two branching gully system in frosted areas and to extract gully morphologic information. Regarding results, at the gully network scale, ALS data had provided accurate maps- the best available- with robust detection of small gullies except where they are narrow or parallel and closely spaced. Mohammadi Torkashvand and Nikkami (2005) concluded that the integration of three data layers (land use, rocks erodibility and land units) in GIS is a suitable method in providing erosion features map such as gully erosion. Mohammadi Torkashvand (2008) differentiates photomorphic units on satellite images as homogenous units with the view of gully erosion and introduced it with data layers integration as the best methods in providing gully erosion map. The aim of this paper is to prepare soil gully erosion map by the supervised classification of satellite images in two lands type including mountainous and plain physiographies.

2. Materials and Methods

Satellite images of ETM$^+$ sensor (year 2004) was used for supervised classification of gully erosion. For investigation of the possibility supervised classification of gully erosion, 3 lands type were considered including:

1. A square-form part of bare low lands in Varamin plain, Tehran province, with 14094 ha located between $51^\circ 43'$ E and $52^\circ 00'$ E, $35^\circ 14'$ N and $35^\circ 20'$ N (basin 1),
2. Above lands (low lands) mixture with cultivated lands located between $51^\circ 44'$ E and $52^\circ 02'$ E, $35^\circ 15'$ N and $35^\circ 21'$ N with 29303 ha area (basin 2),
3. The Roodbar sub-basin, Guilan province, with 102,898 ha located between $49^\circ 15'$ E and $49^\circ 51'$ E, $36^\circ 43'$ N and $37^\circ 02'$ N (basin 3) has been considered as a basin in mountainous land type. Land uses were agriculture lands, rangelands, forests, woodlands and olive orchards. This basin is a part of Alborz mountains strain.

Image processing included radiometric correction, selecting best bands for making color composite with regard to the O.I.F. [1], making principal components 1, 2 and 3, resampling spectral bands and principal components to the panchromatic bands, georeferencing by the nearest neighbour method, making different color composites using the spectral bands, and linear stretching and filtering in different stages for preparation of color composites. All color composites were compared and the best color image was selected for the distinction of gully erosion. From DEM, a hill shade layer was prepared and overlayed on a color composite that obtained 3-D view possibility. Investigations show the possibility visual distinction of more gullies such as small and medium gully erosion on the satellite image is not possible, therefore, by using optimum index factor, 3 bands that had least common information were selected. Therefore, bands 5, 3 and 1 were combined to make a color composite 531 RGB, of course, at first these bands extended by linear stretching.

The position of 652 and 124 ground control points were recorded by GPS respectively in mountain and plain land types. Soil gully erosion, land uses or plant covers were investigated in these points. Regarding ground control points and auxiliary points, training points of gully erosion and other surface features were introduced to software (Ilwis 3.3 Academic). The supervised classified map of gully erosion was prepared by maximum likelihood method and then, overall accuracy of gully erosion map was computed.

3. Results and Discussion

It should be regarded that the visual detection of large gullies is not difficult on images, but there is a problem in distinguishing small gullies. Previously, it had been talked that processing ETM$^+$ images for distinguishing gully erosion intensities were done, but this processing was not caused to detect the small and medium gullies. Hajigholizadeh (2005) also for providing surface, rill and gully erosion maps in five basins in Tehran province, Iran, by using images visual interpretation, concluded that recognition of surface, rill and small gully erosion is very difficult with due attention to images resolution. An applied hypothesis in this study was the possibility of satellite images classification regarding spectral reflexes for detection of gully erosion. This hypothesis can be considered with regards to gully sidewalls angle and change in its spectral reflex than environs. Table 1 shows the results of the supervised classification of gully erosion in plain physiography. In basin 1 that is a homogenous plain with bare lands, accuracy was 85.9%. When these lands were accompanied with cultivated lands (basin 2), accuracy decreased to 59.4%. Decrease in accuracy is because of cultivated lands. This is caused to digital number interference between gullies and cultivated lands. DN difference between gullies and environs is very obvious in a homogenous plain.

[1] Optimum Index Factor

With the current availability of high-resolution satellites such as IKONOS and QuickBird options for detecting and monitoring individual small-scale features have increased, although not yet reported in literature. The visual interpretation provided usually good results and despite of intensive development of numerical interpretation approaches, it is still popular. It is used mainly for erosion mapping of large areas in third world countries (Tripathi and Rao, 2001; Sujatha *et al.,* 2000).

Table 1. Results of the supervised classification of soil gully erosion in plain land type

	Total classified pixels	Classified correct pixels	Accuracy (%)
Gully erosion in basin 1	456	392	85.9
Gully erosion in basin 2	687	408	59.4

Table 2 shows the supervised classification results of gully erosion and other surface features in mountainous physiography. The greatest accuracy is related to forest land use but this value is only 49.8% for gully erosion. Therefore, results denote to a low accuracy in providing gully erosion map that is not acceptable. Previous studies of Mohammadi Torkashvand (2008) indicated that the photomorphic units' map derived from processing satellite images had a 89.9% conformity than ground truth map of gully erosion.

Table 2. Results of the supervised classification of soil gully erosion and other surface features in mountainous land type (Roodbar sub-basin)

Surface feature	Total classified pixels	Classified correct pixels	Accuracy (%)
Gully	325	162	49.8
Agriculture lands	432	314	72.7
Range lands	1120	854	76.3
Forest	2587	2485	96.1
Plant cover	925	764	71.7
Urban	54	48	88.9
		Overall Accuracy: 85.0	

Raoofi *et al.* (2004) computed an accuracy 80% of gully erosion map derived from visual interpretation of Cosmos images than ground truth map. The highest spectral reflexes interference of gully erosion exists with range lands and then agriculture lands. From 325 ground control points (pixel) having gully erosion introduced to software, only 162 pixels had correctly been classified in supervised classified map; 102 and 42 pixels were also classified in range lands and agriculture lands categories, respectively.

Investigations in basin 3 (Roodbar sub-basin) indicated that gullies size in mountain regions are mostly not required size that their pattern be detectable on satellite image. Even when depth and sidewall slope of a gully be high until because of change in spectral reflex to differentiate its DN, again is not required size that be dominant feature at a pixel 28×28 m. We know that the spectral reflex of a pixel is a mean DN of its different features and when a feature is dominant, it influence pixel DN. Therefore, gullies are maybe detectable in a homogenous plain with very low change in slope and relief because of its DN difference than environ that is very obvious, but in mountainous regions with great variations in slope, natural relief, contrast and small size of gullies, there isn't this possibility for supervised classification of gully erosion. When we have a ground control point with distinct coordinate of gully erosion, this pixel isn't introducer of gully erosion, unless gully erosion is dominant in this pixel. Since gullies are linear features and generally distant (the classification of gully erosion is regarding gullies size and its distant), DN of a pixel is only not related to gully erosion.

4. Conclusion

In general, results showed that the possibility supervised classification of gully erosion isn't possible, although it need more studies for results generalization to other mountainous regions. Also, when land uses and other surface features to increase in plain physiography, it will be decreased the classification of accuracy.

Acknowledgements:

Authors are grateful to the Islamic Azad University-Rasht Branch, Rasht, Iran for financial support to carry out this work.

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12/2/2010

Design, Synthesis, and Docking Studies of Novel Diarylpyrazoline and Diarylisoxazoline Derivatives of Expected Anti-inflammatory, and Analgesic Activities

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Abstract: Two series of novel non acidic 3, 5-diarylpyrazoline and 3, 5-diarylisoxazoline derivatives were designed to be synthesized and screened for anti-inflammatory and analgesic activities. In addition, molecular modelling and docking of the designed compounds into cyclooxygenase II (COX-II) using Molsoft ICM 3.4-8C program was performed in order to predict the affinity and orientation of the designed compounds at the active site compared with its binding inhibitor celecoxib. The ICM score values show good agreement with predicted binding affinities, where all the designed compounds exhibit ICM score values (range from -88.89 to -70.40) less than celecoxib (-60.71) revealing higher binding affinity with the enzyme. Accordingly, synthesis of the designed compounds *via* reaction of various propenone derivatives with hydrazine hydrate, phenyl hydrazine or hydroxylamine hydrochlorides were carried out. Evaluation of their activity as anti-inflammatory and analgesics using dextran-induced rat paw edema, formaldehyde arthritis test and paw pressure test, respectively and their ability to induce gastric toxicity was also estimated. All the synthesized compounds exhibited significant activity as anti-inflammatory and analgesic, where compounds **2** and **8** were the most active as anti-inflammatory in dextran-induced rat paw edema, while compounds **7** and **10** were the most active as anti-inflammatory in formaldehyde-induced arthritis rat paw edema test. All compounds showed analgesic activity with the most potent compounds were **3**, and **10** were the most active. No one of the tested compounds cause gastric toxicity. We can conclude that the synthesized compounds proved a successful hit and seem potentially attractive as anti-inflammatory and analgesic agents.
[Gehan Hegazy Hegazy, Ghaneya Sayed Hassan, Nahla Ahmed Farag, Amal Yousef. **Design, Synthesis, and Docking Studies of Novel Diarylpyrazoline and Diarylisoxazoline Derivatives of Expected Anti-inflammatory, and Analgesic Activities.** Life Science Journal. 2011; 8(1): 387-396]. (ISSN: 1097–8135). http://www.lifesciencesite.com.

Keywords: Diarylpyrazoline, Diarylisoxazoline, Anti-inflammatory**,** cyclooxygenase II inhibitor, analgesic, docking, Internal Coordinate Mechanics (ICM).

1. Introduction:

Non steroidal anti-inflammatory drugs (NSAIDs) are the most important class of widely used therapeutics for the treatment of inflammation and pain. The principle pharmacological effects of NSAIDs arise from their inhibition of cyclooxygenases (COXs). Cyclooxygenases control the complex conversion of arachidonic acid to prostaglandins and thromboxanes, which trigger as autocrine and paracrine chemical messengers many physiological and pathophysiological responses [1-3]. The discovery of a second isoform of cyclooxygenase namely COX-II has opened a new line of research based on the assumption that pathological prostaglandins (PGs) are produced by the inducible isoform COX-II while physiological prostaglandins are produced by constitutive isoform COX-I [4]. These physiological protective PGs preserve the integrity of the stomach lining and maintain normal renal function in compromised kidney [5]. The separation of therapeutic effects from the side effects has been a major challenge in the design and synthesis of these drugs. A common structural feature of many selective COX-II inhibitors is the presence of two vicinal aryl rings attached to a central five member heterocyclic moieties [6]. Also, most of the side effects of (NSAIDs) are mainly due to inhibition of both isomers COX-I and COX-II, yet they may also relate to their acidic characters due to the presence of free carboxylic acid moiety [7]. Decreasing acidity or producing non acidic derivatives will solve a part of this problem. Moreover 2- pyrazoline derivatives have been reported to exhibit various pharmacological activities as antimicrobial [8-10] and anti-inflammatory [11-13]. Promoted with the above mentioned studies, it is intended in the present work to investigate the synthesis of novel non acidic 3,5-diarylpyrazoline or 3,5-diarylisoxazoline derivatives adopting facile synthetic approach and utilizing easily accessible starting materials. On the other hand computer docking technique plays an important role in the drug design as well as in the mechanistic study by placing a molecule into the binding site of the target macromolecule in a non-covalent fashion [14].

Molsoft [15] as flexible docking program enable us to predict favourable protein-ligand complex structures with reasonable accuracy and speed. The docking technique will undoubtedly continue to play an important role in drug discovery [16]. So, we docked the designed compounds into cycloxygenase II (COX-II) [17] active site in order to predict their binding modes, their binding affinities and orientation of the designed compounds at the active site of the cycloxygenase II enzyme.

2. Experimental protocols

2.1. General remarks:

All chemicals were obtained from Aldrich (Stenheim, Germany) or Merck chemical Co. (Darmstadt, Germany). Melting points were determined on electrothermal Griffin apparatus (London, UK) and are uncorrected. Microanalysis was carried out at the microanalytical centre, Cairo University, and is within ± 0.4% unless otherwise stated. IR spectra were determined using potassium bromide discs on Shimadzu IR-435 spectrometer (Kyoto, Japan). 1HNMR spectra were made on Joel NMR Varian Gemini 200 MHz spectrometer (Joel, Tokyo, Japan). Chemical shifts (δ) are given in parts per million (ppm) down field from TMS as the internal standard. Mass spectra were recorded on Hewlett Packard 5988 spectrometer at 70 eV (Hewlett-Packard, Palo Alto, CA, USA).

2.2. Chemistry

2.2.1. General procedure for preparation of 5-aryl- 3-(4-bromophenyl)-4,5-dihydro-*1H*-pyrazole **2** and **3**.

A mixture of appropriate propenone **1a-d** (10.0 mmol) and hydrazine monohydrate (99%) (1.0 g, 1.0 mL, 20.0 mmol) was heated at reflux for 6 hr in absolute ethanol (50 mL). The solution was left to cool at room temperature and the solid formed was filtered off, washed with water, dried and crystallized from absolute ethanol.

2.2.1.1. 3-(4-Bromophenyl)-4,5-dihydro-5-(4-methoxyphenyl)-*1H*-pyrazole **2**.

Yield: 67%; m.p: 256-257°C. IR (cm^{-1}): 3220 (NH). ^{1}H-NMR (DMSO-d_6, D_2O) δ ppm: 3.57 (s,3H,OCH_3) , 4.75 (t,1H,CH-NH), 4.98 (d,2H,CH_2), 8.50 (s,1H,NH exch.), 7.21-7.87 (m,8H,Ar). MS: m/z 330 [M^+], 332 [M^++2]. Anal.Calcd. for $C_{16}H_{15}BrN_2O$ (331.21): C, 58.02; H, 4.56; N, 8.46. Found: C, 57.79; H, 4.49; N, 8.81.

2.2.1.2. 3-(4-Bromophenyl)-4,5-dihydro-5-(4-methylphenyl)-*1H*-pyrazole **3**.

Yield: 60%; m.p: 274-276°C. IR (cm^{-1}): 3300 (NH). ^{1}H-NMR (DMSO-d_6, D_2O) δ ppm: 2.35(s,3H,CH_3) , 4.50 (t,1H,CH-NH), 4.90 (d,2H,CH_2), 5.37(s,1H,NH exch.), 7.17-7.51 (m,8H,Ar). MS: m/z 314 [M^+], 316 [M^++2]. Anal.Calcd. for $C_{16}H_{15}BrN_2$ (315.21): C, 60.97; H, 4.76; N, 8.88. Found: C, 60.76; H, 4.60; N, 8.61.

2.2.2. General procedure for preparation of 5-aryl-3-(4-bromophenyl)-1-phenyl-4,5-dihydro-1-phenyl-*1H*-pyrazole **4-6**.

A mixture of appropriate propenons **1a-d** (10.0 mmol) and phenyl hydrazine (1.08 g, 1.0 mL, 10.0 mmol) was heated at reflux for 8 hr in acetic acid. The solution was then cooled and ice was added .the solid formed was filtered off, washed with water and crystallized from ethanol.

2.2.2.1. 3-(4-Bromophenyl)-4,5-dihydro-5-(4-methoxyphenyl)-1-phenyl-*1H*-pyrazole **4**.

Yield: 57%; m.p: 250-252°C. ^{1}H-NMR (DMSO-d_6) δ ppm: 3.45 (s,3H,OCH_3), 3.85 (t,1H,CH-N), 4.75 (d,2H,CH_2), 6.40-7.50 (m,13H,Ar). Anal.Calcd. for $C_{22}H_{19}BrN_2O$ (407.30): C, 64.87; H, 4.70; N, 6.88. Found: C, 64.70; H, 4.52; N, 6.60.

2.2.2.2. 3-(4-Bromophenyl)-4,5-dihydro-5-(2-hydroxyphenyl)-1-phenyl-*1H*-pyrazole **5**.

Yield: 68%; m.p: 130-132°C. IR (cm^{-1}): 3450 (OH). ^{1}H-NMR (DMSO-d_6, D_2O) δ ppm: 4.55 (t,1H,CH-N), 4.65 (d,2H,CH_2), 6.67-7.76 (m,13H,Ar), 8.80 (s,1H,OH exch.). Anal.Calcd. for $C_{21}H_{17}BrN_2O$ (392.8): C, 64.13; H, 4.36; N, 7.12. Found: C, 64.00; H, 4.20; N, 7.01.

2.2.2.3. 3-(4-Bromophenyl)-4,5-dihydro-5-(4-methylphenyl)-1-phenyl-*1H*-pyrazole **6**.

Yield: 75%; m.p: 260-261°C. ^{1}H-NMR (DMSO-d_6) δ ppm: 2.49 (s,3H,CH_3), 3.88 (t,1H,CH-N), 4.85 (d,2H,CH_2), 6.67-7.53 (m,13H,Ar). Anal.Calcd. for $C_{22}H_{19}BrN_2$ (391.3): C, 67.53; H, 4.89; N, 7.16. Found: C, 67.60; H, 4.70; N, 6.92.

2.2.3. General procedure for preparation of 5-aryl- 3-(4-bromophenyl)-4,5-dihydroisoxazole **7-10**.

A mixture of appropriate propenone **1a-d** (10 mmol), hydroxylamine hydrochloride (0.7 g, 10 mmol) and sodium hydroxide (1.0 g) was refluxed in ethanol (50 mL) for 5 hr. The mixture then cooled and solution of diluted ammonium hydroxide was then added drop wise till complete precipitation. The precipitate is filtered and crystallized from ethanol.

2.2.3.1. 3-(4-Bromophenyl)-5-(4-chlorophenyl)-4,5-dihydroisoxazole **7**.

Yield: 74%; m.p: 295-297°C. ^{1}H-NMR (DMSO-d_6) δ ppm: 4.62 (t,1H,CH-O), 4.88 (d,2H,CH_2), 7.16-7.52 (m,8H,Ar). Anal.Calcd. for $C_{15}H_{11}BrClNO$ (336.61): C, 53.52; H, 3.29; N, 4.16. Found: C, 53.70; H, 3.24; N, 4.30.

2.2.3.2. 3-(4-Bromophenyl)-4,5-dihydro-5-(4-methoxyphenyl) isoxazole **8**.

Yield: 81%; m.p: 293-295°C. ^{1}H-NMR (DMSO-d_6) δ ppm: 3.45 (s,3H,OCH_3), 3.85 (t,1H,CH-O), 4.75 (d,2H,CH_2), 7.23-7.80 (m,8H,Ar). MS: m/z 332 [M^+], 333 [M^++2]. Anal.Calcd. for $C_{16}H_{14}BrNO_2$ (332.19):

C, 57.85; H, 4.25; N, 4.22. Found: C, 58.19; H, 4.38; N, 4.00.

2.2.3.3. 3-(4-Bromophenyl)-4,5-dihydro-5-(4-methylphenyl)- isoxazole **9**.

Yield: 64%; m.p: 218-220°C. ^{1}H-NMR (DMSO-d_6) δ ppm: 2.50 (s,3H,CH_3), 4.40 (t,1H,CH-O), 4.60 (d,2H,CH_2), 7.10-7.46 (m,8H,Ar). MS: m/z 316 [M^+], 318[M^++2]. Anal.Calcd. for $C_{16}H_{14}BrNO$ (316.19): C, 60.78; H, 4.46; N, 4.43. Found: C, 61.14; H, 4.46; N, 4.70.

2.2.3.4. 3-(4-Bromophenyl)-4,5-dihydro-5-(2-hydroxyphenyl)- isoxazole **10**.

Yield: 82%; m.p: 320°C. IR (cm^{-1}): 3420 (OH). ^{1}H-NMR (DMSO-d_6, D_2O) δ ppm: 3.80 (d,2H,CH_2), 3.83 (t,1H,CH-O), 7.09-7.81 (m,8H,Ar), 8.49 (s,1H,OH exch.). Anal.Calcd. for $C_{15}H_{12}BrNO_2$ (318.17): C, 56.62; H, 3.80; N, 4.40. Found: C, 56.21; H, 3.80; N, 4.72.

2.3. Pharmacological testing

2.3.1. Anti-inflammatory activity.

All animals use procedures complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee.

2.3.1.1. Dextran-induced rat paw edema:

Edema was induced by injecting 0.1 mL dextran (4% w/v) into the subplanter region of the left hind paw one hour after the oral administration of the tested compound in a dose 100 mg/kg [19]. Paw volume was measured immediately before, 1, 3, and 5 hours after dextran injection using digital plethysmograph (table 1).

2.3.1.2. Formaldehyde arthritis test.

Each test compound was administrated orally at dose of 25, 50 and 100 mg/kg one hour prior to formaldehyde injection and continued for the duration of the experiment [20]. Arthritis was induced by injecting 0.1 mL formaldehyde solution (2% w/v) into the subplanter region of the left hind paw of the rats. The mean change in the paw volume of each treated group was measured at day 3, 5, 7 and 9 by using digital plethysmograph. At the end of the experiment, the animals were sacrificed and the stomach was excised and inspected for haemorrhage and erosions (table 2).

2.3.2. Analgesic activity.

Nociceptive Test (Paw Pressure Test), during all the experiments, the evaluation of antinociceptive effects was carried out using the paw pressure test (Randall and Selitto method) [21]. Increasing pressures measured with an analgesimeter (tip diameter of the probe, 1 mm; weight, 30 g; Apelex; Ugo Basile, Comerio, Italy) were applied to the left hind paw of rats, with a cut-off at 300 g. Vocalization thresholds, considered nociceptive thresholds, were expressed in grams (baseline predrug vocalization thresholds range from 284 ± 28 g to 316 ± 25 g). . An analgesimeter, with a cone-shaped paw pressure and a round tip which applies a pressure of increasing intensity to punctiform area on hind paw of the rat was used. The weight in grams required to elicit nociceptive responses such as paw flexion (reflux withdrawal) of the paw was taken to be the nociceptive threshold. Rats were divided into fifteen groups (n= 6). All tested compounds were given orally at dose of 50 mg/kg. Indomethacin and celecoxib were used as standards. Indomethacin treated group receive a dose 10mg/kg; while celecoxib treated group received 40 mg/kg (table 3).

2.3.3 Acute ulcerogenicity studies

Acute ulceroginicity screening was done according to method reported by Cioli *et al* [22]. The mucosal damage was examined by means of an electron microscope. For each stomach specimen, the mucosal damage was reported.

2.4. Drug modelling studies

All docking studies were performed using "Internal Coordinate Mechanics (Molsoft ICM 3.4-8C)". ICM docking is probably the most accurate predictive tool of binding geometry today [14-17].

Preparation of small molecule

A set of diaryl pyrazoline, and diaryl isoxazoline analogues designed to inhibit cyclooxygenase II was compiled by us earlier; Chem Draw 3D structures were constructed using Chem 3D ultra 8.0 software [Molecular Modelling and Analysis; Cambridge Soft Corporation, USA (2004)], and then they were energetically minimized by using MOPAC (semi-empirical quantum mechanics), Jop Type with 100 iterations and minimum RMS gradient of 0.01, and saved as MDL Mol File (*.mol).

Generation of Ligand and Enzyme Structures

The crystal structure of target protein cyclooxygenase (1CX2) is a COX-II [17] was retrieved from the Protein Data Bank (http://www.rcsb.org/pdb/welcome.do). All bound waters ligands and cofactors were removed from the protein. The amino acids of the binding site where defined using data in pdbsum (http://www.ebi.ac.uk/thorontonsrv/databases/pdbsum

Docking using Molsoft ICM 3.4-8C program

Conversion of our PDB file into an ICM object involves addition of hydrogen bonds, assignment of

atoms types, and charges from the residue templates, then perform ICM small molecule docking through setup the receptor, review and adjust binding site make receptor maps, then start docking simulation, followed by displaying the results. ICM stochastic global optimization algorithm attempts to find the global minimum of the energy function that include five grid potentials describing interaction of the flexible ligand with the receptor and internal conformational energy of the ligand, during this process a stack of alternative low energy conformations is saved. All inhibitors were compared according to the best binding free energy (minimum) obtained among all the run.

3. Results and discussion

3.1. Chemistry

The synthetic pathways utilized to prepare the target compounds are illustrated in scheme 1. Propenone derivatives **1a-d** were synthesized by a base catalyzed Claisen-Schmidt condensation reaction of *p*-bromoacetophenone and substituted aromatic aldehydes in presence of 10% sodium hydroxide in ethanol [18]. Refluxing propenones **1a-d** with hydrazine monohydrate (99%) in absolute ethanol or with phenyl hydrazine in acetic acid afforded the corresponding pyrazolines **2, 3** or phenyl pyrazolines **4-6** respectively. While, reaction of propenones **1a-d** with hydroxylamine hydrochloride yielded isoxazoline derivatives **7-10**. Spectral data (IR, ^{1}H-NMR and MS) of all the newly synthesized compounds were in full agreement with the proposed structures.

3.2. Anti-inflammatory activity

All the newly synthesized compounds **2-10** were evaluated for their anti-inflammatory activity using dextran-induced rat paw edema method [19]. The tested compounds and reference drugs were administered orally at a dose of 100 mg/kg one hr before dextran injection into the subplanter region of the left hind paw. Paw volume was measured immediately before and 1, 3, 5 hr after dextran injection by using digital plethysmograph. Results are listed in table 1 and illustrated in figure 1-A. Where, compounds **2** and **8** were the most active. The anti-inflammatory activity was also measured using formaldehyde arthritis test [20]. Arthritis was induced by formaldehyde injection into subplanter region of the left hind paw of the rats; the mean change in the paw volume of each treated group was measured at day 3, 5, 7 and 9 by using digital plethysmograph. The results are listed in table 2 and illustrated in figure 1-B. It was found that all the synthesized compounds possess significant anti-inflammatory activity, where compounds **7** and **10** were the most active.

3.3. Analgesic activity

Paw pressure test was carried out according to the Randall and Selitto test [21] which is based on determination of the animal threshold response to pain induced in the paw by the application of a uniformly increasing pressure. The results are listed in table 3 and illustrated in figure 1-C. It was found that all the synthesized compounds show good analgesic activity and compounds **3** and **10** were the most active.

3.4. Acute ulcerogenicity studies

All the synthesized compounds were subjected to ulcerogenicity potential test [22] at 12 times the therapeutic dose of diclofenac with additional physical (cold) stress for 2 hr at -20 ^{o}c. Ulcerogenic effect of the synthesized compounds in animal efficacy model was evaluated for gastric ulcerogenic potential in rat stress model. When compared with diclofenac, all the compounds did not cause any gastric ulceration at the above mention doses. Hence gastric tolerance to these compounds was better than that of diclofenac.

3.5. Docking studies

To understand the pharmacological data on structural basis, we evaluate the designed compounds (three different classes of diarylpyrazoline, triarylpyrazoline and diarylisoxazoline) through docking techniques using Molsoft ICM 3.4-8C program. We docked our designed compounds on one of the crystal structures of cyclooxygenase II available through the RCSB Protein Data Bank (PDB entry 1CX2) [17]. The scoring functions of the compounds were calculated from minimized ligand protein complexes. In order to compare the binding affinity of the newly synthesized diarylpyrazoline and diarylisoxazoline analogues, we docked compounds **2 -10** into the empty binding site of cyclooxygenase II with its bound inhibitor celecoxib (1CX2), figures 2a-c show the docking solutions with the highest predicted binding affinity for cyclooxygenase II. Figure 2a shows orientation of celecoxib, while figures 2b and 2c show orientations of compounds **2** and **4** respectively. As shown from the (tables 1-4, figures 2a-c) the following results can be drawn: The ICM score values show good agreement with predicted binding affinities obtained by molecular docking studies as verified by pharmacological screening. Where the designed compounds shows ICM score values (range from -88.89 to -70.40) less than celecoxib (-60.71) revealing higher binding affinity with the enzyme table 4.

Celecoxib (the original ligand) reveals ICM score of -60.71 and form seven hydrogen bonds with Tyr355, His90, and Arg513 (table 4, figure 2a).

Diarylpyrazoline derivatives **2** and **3** show relatively high binding affinity, where compound **2** has ICM score of –77.98 and form one hydrogen bond with Ser530 (table 4, figure 2b), while compound **3** exhibit ICM score of -75.77 without hydrogen bond but one of its conformers has a score of -68.16 and form three hydrogen bonds with His90, Arg513 (table 4). Triarylpyrazoline derivatives **4, 5** and **6** exhibit lesser score values relatively to diaryl derivatives revealing higher binding affinity, compounds **4** possess ICM scores of -87.02 and form four hydrogen bonds with Arg120, Tyr355 (table 4, figure 2c), while compound **5** has ICM score of -88.67 and form one hydrogen bond with Val523, and compound **6** exhibit ICM score of -88.89 without hydrogen bond but one of its conformers has a score of -79.67 and form one bond with Tyr355. Diarylisoxazoline compounds **7, 8, 9**, and **10** possess ICM scores ranges from –81.71 to -70.40, where compound **7** has ICM score of -79.76 and form two hydrogen bonds with Arg120, and compound **8** possesses ICM score of -79.41 and forms three bonds with Ala378, and Asp125 and compound **9** has ICM score of -70.40 with no hydrogen bond while another conformer has ICM score of -69.44 and form six bonds with His90, Arg513. Finally, compound **10** which has the most anti-inflammatory effect possesses ICM score of -81.71 and form one hydrogen bond with Asn375, where another conformer possesses ICM score of -78.39 and form eight hydrogen bonds with His90, Ser530, and Arg513 (table 4).

Table 1: Effect of the test compounds on dextran-induced rat paw edema compared with indomethacin and celecoxib.

Compound	Edema volume (mL)		
	1 hr	3 hr	5 hr
Control	0.86±0.10	1.90±0.32	1.17±0.21
Indomethacin	0.67±0.11	0.71±0.12*	0.51±0.13*
Celecoxib	0.71±0.11	1.44±0.51*	0.74±0.22*
2	0.74±0.09	1.29±0.31	0.80±0.22
3	0.81±0.15	1.44±0.17	0.90±0.31
4	0.78±0.14	1.39±0.61	0.88±0.19
5	0.81±0.15	1.55±0.51*	0.97±0.12*
6	0.76±0.13	1.28±0.45	0.82±0.09
7	0.79±0.07	1.41±0.12	0.87±0.31
8	0.73±0.18	1.2±0.35	0.77±0.08
9	0.83±0.11	1.73±0.38*	1.08±0.22*
10	0.84±0.08	1.77±0.41*	1.09±0.21*

* Significant from control at $p < 0.01$ compared to control using ANOVA followed by Tukey- Kramer as post ANOVA test.

Table 2: Effect of the test compounds on formaldehyde-induced arthritis rat paw edema compared with indomethacin and celecoxib.

Compound	Edema volume (mm)			
	3 days	5 days	7 days	9 days
Control	2.30 ±0.11	3.5 ±0.61	3.20±1.01	3.06±1.11
Indomethacin	1.05±0.17*	1.41±0.08*	1.31±0.07*	1.10±0.60*
Celecoxib	1.17±0.08*	1.66±0.09*	1.60±0.33*	1.24±0.22*
2	2.18±0.41	3.10±0.32	2.97±0.09	2.30±0.71
3	1.53±0.21*	2.27±0.41*	2.14±0.54*	1.70±0.22*
4	1.73±0.18	2.62±0.09*	2.50±0.19	1.80±0.04*
5	1.64±0.71*	2.43±0.62*	2.19±0.13*	1.90±0.32*
6	2.25±0.18	2.45±0.09*	2.40±0.13*	2.30±0.61
7	1.45±0.08*	2.17±0.19*	2.08±0.17*	1.50±0.33*
8	1.58±0.19*	2.38±0.41*	2.30±0.15*	1.61±0.16*
9	2.27±0.28	3.45±0.21	3.09±0.15	2.80±0.01
10	1.42±0.32*	2.00±0.28	1.89±0.41*	1.40±0.11*

* Significant from control at $p < 0.01$ compared to control using ANOVA followed by Tukey- Kramer as post ANOVA test.

Table 3: Study of the analgesic effect of the test compounds using the paw pressure test.

Compound	Weight by gm			% Change in weight		
	1 hr	3 hr	6 hr	1 hr	3 hr	6 hr
Control	201±0.12	204±0.19	204±0.18	-	-	-
Indomethacin	426±0.12*	442±0.29*	298±0.27*	111.94	116.67	46.08
Celecoxib	410±0.13*	448±0.10*	286±0.13*	103.98	119.61	40.20
2	374±0.11*	397.5±0.36*	228±0.16*	86.07	94.85	11.76
3	398±0.21*	432.5±0.25*	231±0.06*	98.01	112.01	13.24
4	321±0.21*	350±0.19*	249±0.27*	59.7	71.57	22.06
5	341±0.17*	374±0.29*	245±0.33*	69.65	83.33	20.10
6	271±0.10*	305±0.61*	242±0.18*	34.83	49.51	18.63
7	211±0.31*	240±0.19*	206±0.29*	4.98	17.65	98.00
8	244±0.21*	275±0.40*	219±0.26*	21.39	34.80	7.35
9	297±0.23*	322.5±0.20*	258±0.35*	47.76	58.09	26.47
10	379±0.13*	402±0.36*	240±0.31*	88.56	97.06	17.65

All compounds are significant from control at 1, 3 and 6 hours at $p < 0.05$ compared to control using ANOVA followed by Tukey- Kramer as post ANOVA test.

Table 4: ICM Scores of celecoxib, the compounds, and hydrogen bonds formed with amino acid residues

Compounds	ICM scores	No. of Hydrogen bonds	Involved amino acid
Celecoxib	-60.71	7	Tyr355, Tyr355, His90, Arg513, Arg513, Arg513, Arg513
2	-77.98	1	Ser530
3	-75.77 or -68.16	0 3	…….. His90, His90, Arg513
4	-87.02	4	Arg120, Arg120, Arg120, Tyr355
5	-88.67	1	Val523
6	-88.89 or -79.67	0 1	…..... Tyr355
7	-79.76	2	Arg120, Arg120
8	-79.41	3	Ala378, Ala378, Asp125
9	-70.40 or -69.44	0 6	…….. His90, His90, Arg513, Arg513, Arg513, Arg513
10	-81.71 -78.39	1 8	Asn375 Ser530, His90, His90, His90, Arg513, Arg513, Arg513, G354

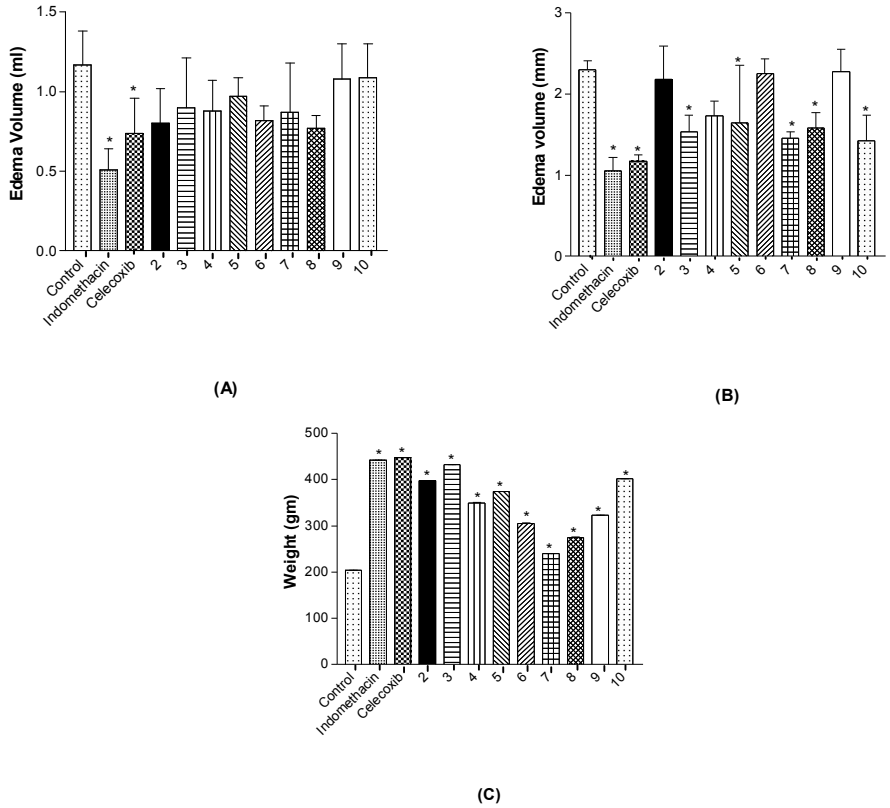


Figure 1: Anti-inflammatory and analgesic effects of diarylpyrazolin and diarylisoxazolin derivatives on (A) dextran-induced rat paw edema after 5 hr; (B) formaldehyde arthritis test after 3 days; (C) paw pressure test after 3 hr. Values are mean of 6 data points ± S.D. *P< 0.01 compared to control group using ANOVA followed by Tukey-Kramer as post ANOVA test.

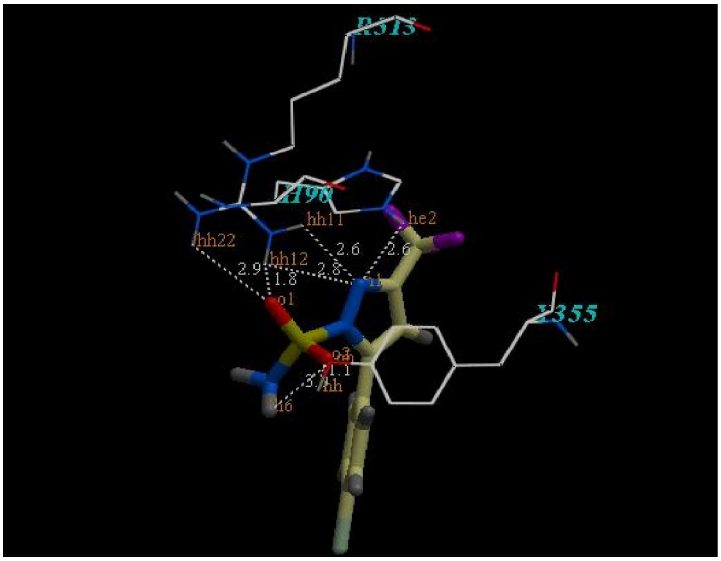


Figure 2a: Binding mode of the original ligand celecoxib into its binding site of cycloxygenase II, it has ICM score -60.71, and form 7 hydrogen bonds shown as white dotted lines (table 4), showing one hydrogen bond between NH of His90 with N1 of pyrazoline moiety distance 2.01 A^o, and two hydrogen bonds between H of OH of Tyr-355 and O of SO_2NH_2, and between O of OH of Tyr355 and H of SO_2NH_2 distances 1.13 A^o and 2.70 A^o respectively, and another four bonds between 4H of 2 NH_2 of Arg513 with N1 of pyrazoline moiety, and 2O of SO_2NH_2 distances 2.41 A^o, 2.54 A^o, 1.74 A^o, and 2.69 A^o respectively.

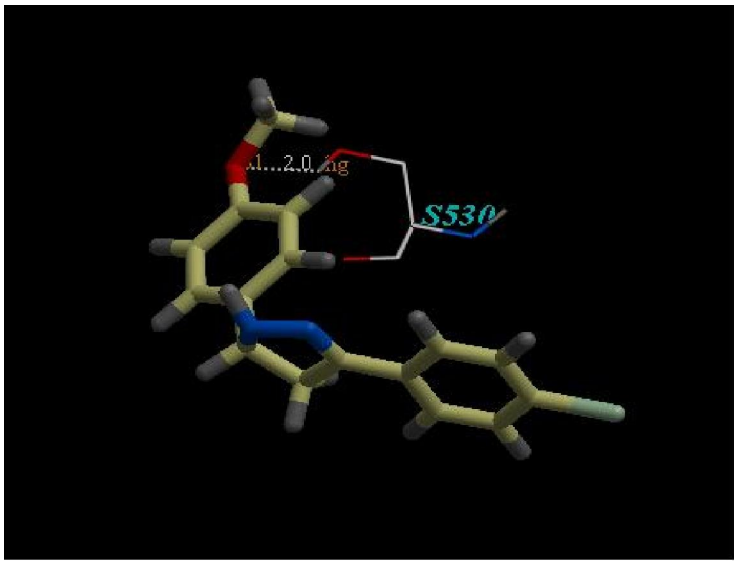


Figure 2b: Binding mode compound **2** into its binding site of cycloxygenase II, it has ICM score -77.98, and form one hydrogen bond shown as white dotted lines (table 4), showing one hydrogen bond between O (OCH_3), with OH of Ser530 distance 1.77 A^o.

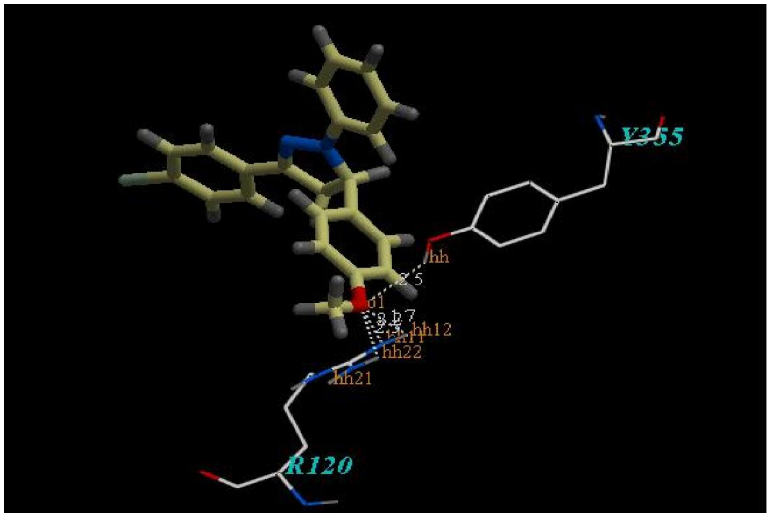
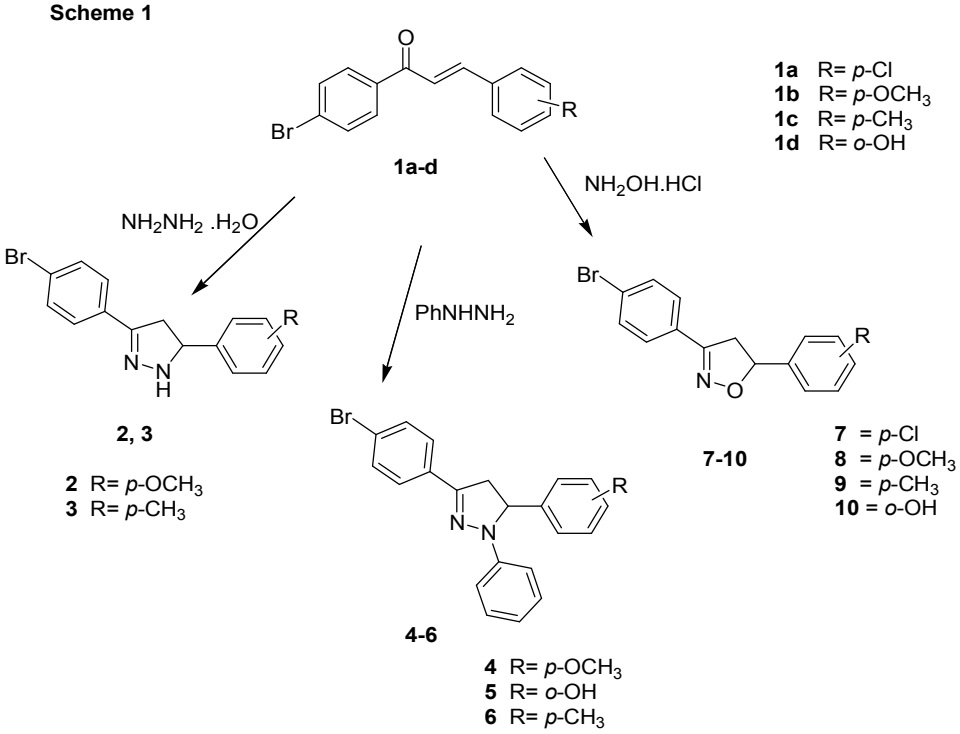


Figure 2c: Binding mode of compound **4** into the binding site of cycloxygenase II, it has ICM score -87.02, and form four hydrogen bonds shown as white dotted lines (table 4), showing three hydrogen bonds between O of (OCH_3), with NH_2 of Arg120 distance 2.74 A^o and 2.09 A^o, and 2.44 A^o respectively, and one bond between O of (OCH_3) and OH of Tyr355 distance 2.03 A^o.

Scheme 1



4. Conclusion

From the previous data we can conclude that acidic group sulfamido which is found in most COX-II inhibitor is not essential for the anti-inflammatory and analgesic activity but it enhances it, where diarylpyrazoline and diarylisoxazoline moieties may be responsible for activity. Compound **8** is the most active one as anti-inflammatory in dextran-induced rat paw edema, while compound **10** is the most active one in formaldehyde arthritis test. Other compounds showed activity ranging from mild to moderate that compared to celecoxib. For the analgesic activity, all the test compounds showed promising activity, where the most active compounds were **3** (diaryl pyrazoline moiety) and **10** showing activity parallel to celecoxib. It is clear that presence of *p*-methoxy group in phenyl ring increased the anti-inflammatory activity of compounds **2, 4** and **8**. To less extent the presence of *p*-methyl group may also increase the activity as shown by compounds **6** and **9**. Furthermore, the presence of *o*-hydroxyl group increases the analgesic and anti-inflammatory activity as shown by compounds **5** and **10**. Also, the smaller the ICM score value and/or formation of hydrogen bonds with Arg120, Arg513 , Ser530, Ser353, Try355, and His90 amino acids lining the pocket of COX-II enzyme, increasing the binding affinity, and hence the anti-inflammatory effect. No one of the tested compounds show ulcerogenic activity.

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12/21/2010

Magnetized water and saline as a Contrast agent to Enhance MRI Images

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Abstract: MRI image enhancements have been carried out using different contrast agents. In this research we started with testing the effect of accurately pre-specified magnetized water on MRI received signal, and then considered the magnetized-saline (MS) as a new MRI brain contrast agent (CA).A 40 years old 80kg male injected with 250ml MS. Couple groups of MRI images were performed over the same circumstantial conditions and MRI protocol; before and after the injection. The focused study on MRI showed a significant difference in image intensities after injecting the MS compared to normal MRI images, and water contour of the white matter in T2 WIS is more obvious than before saline injection series. Further quantitative measurements applied using MATLAB genetic algorithm. Leading to the result; magnetized saline injection affect signal intensity and enhance contrast in MRI brain images.

[Samir M. Badawi, Wael Abou EL-wafa. Ahmed, Yasser M. Kadah. **Magnetized water and saline as a Contrast agent to Enhance MRI Images.** Life Science Journal. 2011;8(1):397-401] (ISSN:1097–8135). http://www.lifesciencesite.com.

Keywords: Image contrast; MRI; magnetized water; enhancement.

1. Introduction

Magnetic resonance imaging (MRI) is one of the most promising non-invasive diagnostic technique in medicine [1]. For an increasing number of studies of internal organs, magnetic resonance imaging (MRI) reached the highest resolution in images and also the fastest acquisition times. MRI is a special tool in this regard, because of its relatively high spatial resolution (10μ m in high magnetic field scanners) and capacity to scan entire organisms noninvasively, since the quality of the MRI images depends on the three NMR main parameters (proton spin density, nuclear spin–lattice relaxation time T1 and spin–spin relaxation time T2, it is easy to imagine the high interest in development of new natural and safe contrast agents (CA) able to enhance images by increasing (locally) the nuclear relaxation rates [2].Most MRI contrast agents are paramagnetic chemicals that increase parameters called the T1 and T2 relaxation rates of water, as observed in tissue and solution; T1 or T2 relaxation enhancements produce image brightening or darkening, respectively.

Additional classes of contrast agent's work by a chemical exchange-based mechanism called chemical exchange saturation transfer (CEST) [3], or involve imaging nonstandard nuclei like 19F and 13C. The characteristics and physical mechanisms of different types of contrast agent are discussed at length in a number of book chapters and reviews [4–9],and are summarized in table(II). In general, for any agent to be used in functional imaging, either its ability to influence MRI contrast or its spatial distribution must be sensitized to neural activity in some way. The Toxicity of contrast agents should be considered, where Nephrotoxicity (toxicity to the kidneys) is a major consideration for clinicians when requesting tests which use an iodine-based contrast media. Patients whose renal function is impaired (usually with a creatinine >120 micro mol/liter) should only have contrast media if absolutely necessary. In these circumstances, a special form of contrast media, which is 'kinder' to the kidneys, can be given to prevent contrast-induced nephropathy [10]. Nephrogenic systemic fibrosis (NSF) with MRI contrast agent can appear through the administration of gadolinium for MR contrast enhancement. Although rare and only in renal compromised patients, it produces serious side- effects that may involve fibrosis of skin, joints, eyes, and internal organs. Because of this toxicity, using magnetized water or injecting magnetic saline will be healthier than normal contrast agents in MRI.

Regarding the magnetized water, Magnet researchers Davis and Rawls found that south-pole magnetic force appeared to make water molecules bind to each other more weakly than normal, thus giving it a lower surface tension than normal. When water is magnetized, some of its physical and chemical properties are altered; density, boiling point, electrical conductivity, surface tension, viscosity and increase the pH, making it more alkaline [15].

2. Material and Methods

- ***Magnetic water phantom Imaging***

Two water phantoms used. Each one is constructed of biodegradable latex rubber balloons and filed with 450 ml. one is field with normal tap water to be used as a reference, where the other is field with magnetic water. Both phantoms are scanned using small body coil of 0.2 Tesla MR (IRIS MATE, Hitachi, Japan). The magnetic water phantom scanned after 4 hours of magnetization figure (1) and figure (2)..

The resulted images for both magnetized and non-magnetized water phantoms are quantitatively processed by MATLAP Genetic Algorithms (GAs) as shown in figure(6).We applied the following signal equation for a repeated spin-echo sequence as a function of the repetition time (TR), and the echo time (TE) where it defined as the time between the 90o pulse and the maximum amplitude in the echo

$$S = k\,(1 - e^{-TR/T1})\,e^{-TE/T2} \quad (1)$$

This equation is only valid when TR >> TE. In our experiment we used TR= 2700,TE=120, and

k = 8560*107

- ***MRI brain imaging***

Brain imaging experiment executed on a volunteer (40 years old, 80 kg, 173 cm) using a standard head coil of 1.5 T MRI machine (Visart, Toshiba, Tokyo, Japan). The initial MRI examination included axial Fast spin-echo (FSE), T2-weighted [repetition time (TR) 4500 ms, echo time (TE) 120 ms], matrix of 160X256. Two identical image series of MRI brain images acquired. The first image series done at 1.00 PM with no IV saline injection, figure (4). Where at 2.00 PM the volunteer injected with 250ml IV MS; and the second image series acquired, figure (5). The volunteer did not report any complaints during or within 48 h after the injection of MS. The MS was developed using permanent magnetic funnel, where considered as a contrast agent (CA) for application in MRI. Each 100 ml IV saline contains:

- Sodium chloride 0.9 G
- Water for injection Q.S.
- Sodium 150 mEq\L
- Chloride 150 mEq\L

3. Results

As the positive results obtained from our experiments, the expert radiologists recommend that the procedure needs to be applied in real cases like abscesses and tumors, then they recommend performing other MRI fluid sensitive techniques as FLAIR (fluid attenuation inverse recovery), the GA in MATLAB show that T1 is Increased to 1.513 s in magnetized phantom instead of 0.672 s in non-magnetized one and T2 did not changed (0.012 s) and S/N increased from 156.3 to 337.5

- ***Phantom Imaging analysis***

Quantitative analyses performed using MATLAB Genetic algorithms (GAs) as shown in figure (6) to estimate T1 and T2 for both magnetized and non-magnetized water phantoms based on signal to noise ratio for both images. Table (1) shows imaging parameters in addition to S/N ratio, and results of GAs. We used for both magnetized and non-magnetized images the same calculating parameters as: Function tolerance = 1e-100 ,Generation = 10000 The GAs results shows change in T1, where no changes occurred in T2.

- ***Brain imaging analysis***

Qualitative and quantitative analysis considered to insure the research results. The qualitative analyses done by two expert radiologists jointly where they analyzed matched pre-injection and post-injection images. The process carried out based on visual inspection and experience as regular diagnostic and reporting process. Remarkable changes were recorded on post-injection images by the expert radiologists as following:

- Slight increase of the dimensions of the ventricular system & CSF spaces.
- Water contour of the white matter in T2 WIS is more obvious than before saline injection series.

Computer-assisted analysis was performed (quantitative analyses) using the Medical Image Processing, Analyses and Visualization (MIPAV), Center for Information Technology (CIT), National Institutes of Health (NIH), Version: 4.2.1.

As shown in Fig (3) we find a clear changes in the left side figure. the LUT is totally deferent between two images and pixels count is more in magnetized injected images.

4. Conclusions

In conclusion, this study clearly indicated that magnetized injection saline as CA enhances MRI images. We proved that this technique is a clinically healthy and feasible technique for better diagnosis in MRI Imaging because as indicated in many references that Magnetic water is healthy [16-19]

TABLE 1 WATER PHANTOMS IMAGING PARAMETERS AND RESULTS OF MATLAB GAS

Magnetization time	TR	TE	S/N ration	T1	T2
0 Hours	2700	120	**156.3**	672	11
4 Hours	2700	120	**337.5**	1513	12

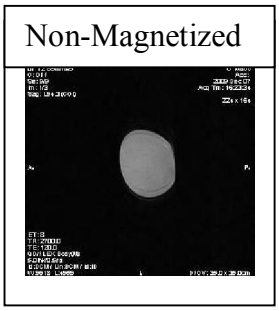

Figure 1. non-magnetized

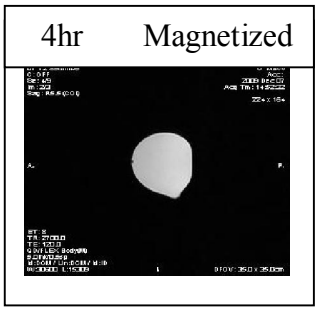

Figure 2. magnetized water

Acknowledgements:

Authors are grateful to Prof.Ehab Reda and Dar alashaa radiology center, Alexandria, Egypt and Dar Al-Fouad Hospital for help and support by using their MRI machines to made our experiments, and also Mr. Mohamed Ramadan, MRI technologist for his cooperation

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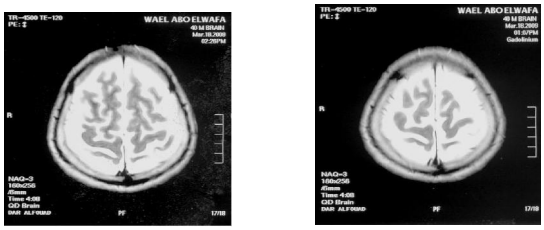
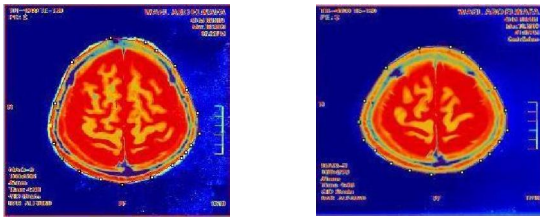
TABLE 2 CHARACTERISTICS AND PHYSICAL MECHANISMS OF DIFFERENT TYPES OF CONTRAST AGENT

(a) Paramagnetic atoms promote T1 relaxation-based contrast in conventional MRI by interacting with water molecules (left). Gadolinium atoms (green) are effective at this because of their high electron spin (S = 7/2); Mn2+ (S = 5/2) and a variety of other metal ions may also be used. Relaxation occurs when water molecules (cyan) sample magnetic field perturbations (yellow) created by the paramagnetic atom,
(b) super paramagnetic nanoparticles including SPIOs have the highest T2 reflexivity, and relatively low T1 reflexivity. SPIOs typically contain a core of iron oxide 3–10 nm diameter (green), surrounded by a biocompatible organic coating with a total diameter of 10–100 nm (gray). Particles induce magnetic perturbations (yellow) that induce relaxation of water molecules diffusing in proximity (blue arrows). The particle size and shape of its field perturbation influence its reflexivity [11]—this relationship is the basis of sensors formed by making SPIO aggregation dependent on presence of a target molecule [12].Addition of a T2 contrast agent causes reduction of the MRI signal (bottom right) and leads to image darkening in areas where the contrast agent is concentrated.
` (c) Chemical exchange saturation transfer (CEST) contrast can be produced using agents with exchangeable protons that have MRI resonance frequencies (chemical shifts) well resolved from the frequency of water molecules [3]. The example shown is the in dole nitrogen proton (indigo) of 5-hydroxytryptophan. The spectrum of chemical shifts in a solution of this agent is schematized by the gray trace at the bottom left, where resonances of the CEST agent protons and water protons are indicated by indigo and cyan arrowheads,
(d) Contrast agents incorporating 13C, 19F, or a variety of other nuclei may be imaged directly using modified MRI hardware. Images of 13C agent distribution may be formed, In experiments of Golman et al. [13], carbon resonances of 13C1-labeled pyruvate (green) and its reduction product 13C1-lactate (gray) could be distinguished using this approach (right). Relative amounts of the two species were indicative of local metabolic rate. Images like the one shown (left) were obtained only with the use of 13C-labeled agents that had been hyperpolarized to boost MRI signal, before imaging [14].

MRI with Magnetized Saline injection

MRI without Magnetized Saline injection





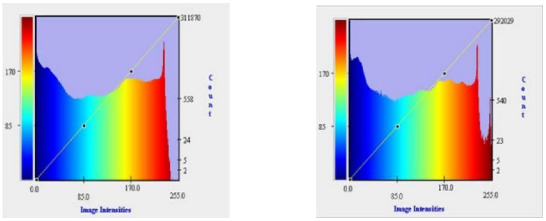


Figure 3. Analysis using medical image processing, analyses and visualization (MIPAV)

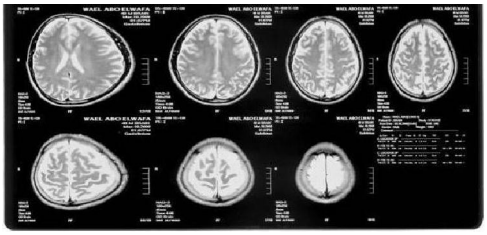


Figure 4. Brain MRI pre-intravenous injection of Magnetized saline

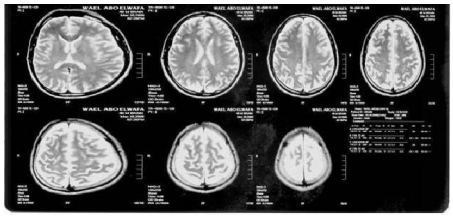


Figure 5 . Brain MRI post-intravenous injection of Magnetized saline

Magnetized Water

Non Magnetized Water

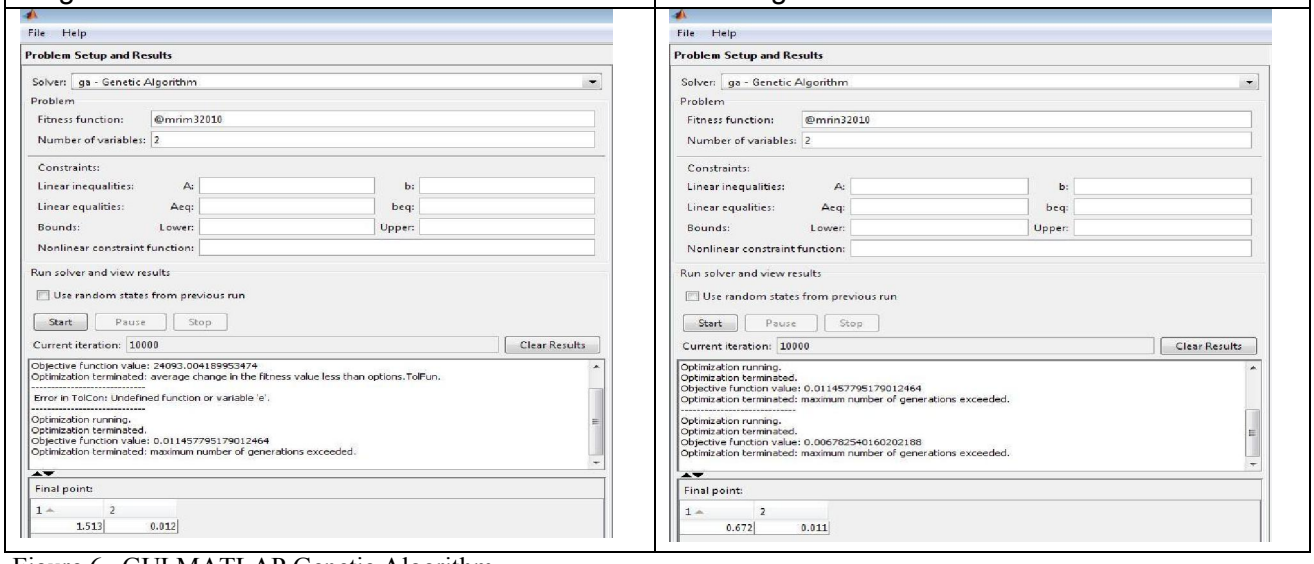


Figure 6 . GUI MATLAP Genetic Algorithm

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1/12/2010

Pathogenicity of Aeromonas on Embryonated Chicken Eggs

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Abstract: Pathogenicity of Aeromonas on embryonated chicken eggs was studied in three species of Aeromonas : (*Aeromonas hydrophila, Aeromonas caviae and Aeromonas sobria*) in embryonated chicken eggs either by inoculation via yolk sac route or dipping of egg incubated for till hatching . The criteria of judgment was mortality , hatchability, reisolation from dead embryo, histopathological changes in liver, yolk sac of dead embryo on hatched chicks Yolk sac inoculation of three species of Aeromonas in a dose 1.5x 10^7/ml gave 100% embryonic mortalities after 3 days and the reisolation from liver 83%, 75%, 50%, respectively and from yolk sac 91.6%,75%,66.6% respectively. In dipped eggs in media contains three Aeromonas species in adose 1.5x10^7/ml gave embryonic mortalities 25%, 33.4%, 17% respectively reisolation rate of Aeromonas species from liver 33.3%, 25%, 0% respectively and from yolk sac 100%, 50%, 100% respectively while hatchability was 75%, 66.6% and 83% respectively. Hatched chicks showed pathological changes in both liver and intestine. Finally the results indicated that, Aeromonas strains *(A.hydrophila, A.caviae, A.sobria*) were highly pathogenic for chicken and causing embryo mortalities and decrease of the hatchability.

[Zeinab. M.S. Amin Girh, K.M. Mahgoub, Nagwa Rabie, S.,; Sahar A. Zoo El-Fakar and M.A. Kutkat. **Pathogenicity of Aeromonas on Embryonated Chicken Eggs.** Life Science Journal. 2011;8(1):402-407] (ISSN:1097–8135). http://www.lifesciencesite.com.

Keywords: Pathogenicity, Aeromonas, histopathological, embryonated.

1. Introduction:

Aeromonas is a member of family vibrionaceae ,placed in a genus Aeromonas which include four phenotypical separated species named Aeromonas *hydrophila* (*A. hydrophila), Aeromonas Caviae,(A. Caviae), Aeromonas Sobria(A. Sobria) and Aeromonas Salmonicida (A. Salmonicida)* (Altwegg, 1988).

Aeromonas normally inhabits brackish, Fresh, estuarine, marine, chlorinated and unchorinated water supplies (slotnick, 1970; Kaper et al.,1980; Atkinson, 1986; Van Derkooj, 1988).

Aeromonas produces cytotoxins so it has a public health significance as a potential cause of food – borne illness . (Barnhart and Puncorbo 1992).

Aeromonas hydrophila, either alone or in combination with other organisms, can cause localized and systemic infection in avian species including poultry (Glunder and siegmann 1989, and Shane and Gifford. 1985). Aeromonas was recorded in chickens and turkeys suffering from enteritis and watery feces (Efuntoye, 1995) as well as in ducks suffering from salpingitis , Septicemia and/ or air saculitis (Bisgaard 1995;Li. et al., 1998 and watts, et al., 1993).

No available literature dealing with the transmission of Aeromonas through the ovary (ovo transmission of Aeromonas) while ,Aeromones has been recovered from deed – in – shell embryos and weak chicks (lin – et al., 1996).The contamination of chicken carcasses with motile Aeromonas speices was occurred in the slaughtering process from the intestinal content to carcasses via processing water (Akan et al., 1998; and Sarimeh metoglu and Kupulu, 2001).

This study was aimed to study the pathogenicity effect of Aeromonas on the embryonated chicken eggs (ECE).

2. Material and Methods

Aeromonas Strains

The *A.hydrophila, A.Caviae, A. Sobria* were kindly supplied by (first author)Dr. Zeinab Girh, Poultry Diseases Department National Research Center. These strains were maintained on nutrient agar slant by routine subculture of regular intervals. The strains were stored at 4°C and periodically tested for purity.

Media

Aeromonas ager medium (oxoid) was used for cultivation and propagation of Aeromonas species. The culture was incubated aerobically at 30C^o for 20-24 hours. The typical colonial appearance of Aeromanas species were selected and identified according to Krieg and Holt. (1984) and Havelaar et al. (1992).

Embryonated Chicken Eggs

Nighty six specific pathogen free (SPF)

embryonated chicken eggs (ECE) obtained from kom-Oshim, El – Fayom. Eggs were incubated in egg incubator at 37c° humidity 50 %. Fertile chicken eggs were used for study the pathogenic effect of A. hydrophila, A. caviae, A. sobria on the viability of the chicken embryo and its effect on hatched chicks.

Experiment Design

Aeromonas Strains,A.hydrophila, A. caviae and A. sobria were aerobically grow on Aeromanos agar medium (oxoide) at 30c° for 20-24hr. and resuspended in a concentration of (1.5×10^7) CFU/ML. SPF embryonated chicken eggs were devided into 2 equal groups 48 eggs of each,(groups A and B).

Group A was subdivided to 4 subgroups 12 eggs of each (A1-A4), inoculated via yolk sac at 5 days -old with 0.2ml of the bacterial culture of *A. Hydrophila, A. Caviae and A. Sobria* respectively and sub group A_4 was kept as negative control.

Group B was also Subdivided into 4 subgroup (B1-B4) each dipped in bacterial Culture containing 1.5×10^7/ml of *A. hydrophila, A. Caviae and A. Sobria* while B4 were kept as negative control. ECE were incubated and candled every 24 for recording mortality till hatch. Livers and yolks of the dead embryos from each group were subjected to reisolation of Aeromonas species. The hatched chicks from each group were killed. Livers and intestines were collected for reisolation of Aeromonas spp. and histopathological examination.

Histopathological examination:

Histopathological examination was carried out according to the method of (Shane and Gifford, 1985) Representative samples from livers and intestines of dead embryos were immersed and fixed in 10% formalin saline. These samples were dehydrated, cleared, embedded and cut to 7um then they were transferred to glass slides and stained with hematoxylin and eosin. Then they were examined by ordinary microscope.

3. Results:

Table (1): Mortalities of embryoneted chicken after inoculation with 1.5 x *10^7/ml of A. hydrophila,A. caviae,A.sobria,* via yolk sac route

Group Code	Aeromonas SP	No. of Inoculated Eggs	Days Post inoculation of Aeromonas													
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A1	A.hydrophila	12	3D*	6D*	3 D	-	-	-	-	-	-	-	-	-	-	-	-
A2	A.caviae	12	7D	5D		-	-	-	-	-	-	-	-	-	-	-	-
A3	A.sobria	12	6D	6D		-	-	-	-	-	-	-	-	-	-	-	-
A4	Control	12	1D	1D		-	-	-	-	-	-	-	-	-	-	-	-

*D; Died embryo

Table (2): Mortality of embryoneted chicken eggs after dipping in media containing 1.5 x 10^7/ml of *Aeromonas (A.hydrophila,A.caviae and A.sobria)*

Group Code	Aeromonas Species	No of Eggs	Days Post Dipping in Aeromonas														
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
B1	A.hydrophila	12	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0
B2	A.caviae	12	0	0	0	0	2	0	1	1	0	0	0	0	0	0	0
B3	A.sobria	12	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0
B4	Not inoculated	12	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0

Table (3): Reisolation of *Aeromonas* species from liver and Yolk of dead embryos

Group Code	Aeromenas spp.	Total No of Eggs	No of Dead embryo	Reisolation of Aeromonas species from internal organ			
				Liver	%	Yolk	%
1A	A. hydrophila	12	12	10	83	11	91.6
2A	A. caviae	12	12	9	75	9	75
3A	A. sobria	12	12	6	50	8	66.6
4A	Control	12	0	0	0	0	0
1B	A. hydrophila	12	3	1	33.3	3	100
2B	A. caviae	12	4	1	25	2	50
3B	A. sobria	12	2	-	0	2	100
4B	Control	12	3	0	-	0	-

Table (4): Reisolation of *Aeromanos species* from liver and Yolk of hatched chicks hatchability Percentage

Group Code	Aeromenas Species	Total No ,of Eggs	No, of Hatched embryo	Hatchability %	Site of reisolation			
					Liver	%	Yolk	%
B1	*A.hydrophila*	12	9	75	4	44.4	6	66.6
B2	*A.caviae*	12	8	66.6	4	50	5	62.6
B3	*A.sobria*	12	10	83	1	10	3	30
B4	Not treated	12	9	75	0	-	0	-

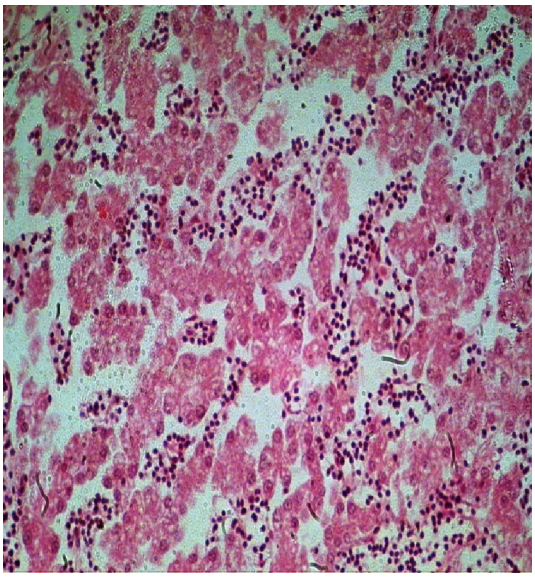

Fig (1): liver showing sever congestion in the hepatic blood vessels and sinosoids they were distended and encorged with blood.

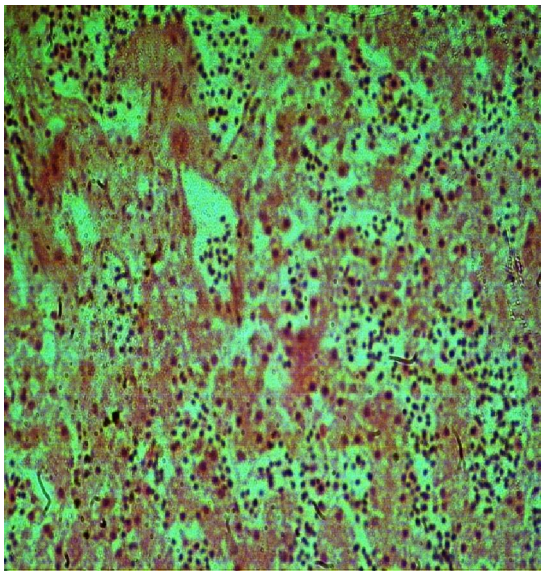

Fig. (2): liver showing focal areas of coagulative necrosis with disturbance in the arrangement of hepatocytes.

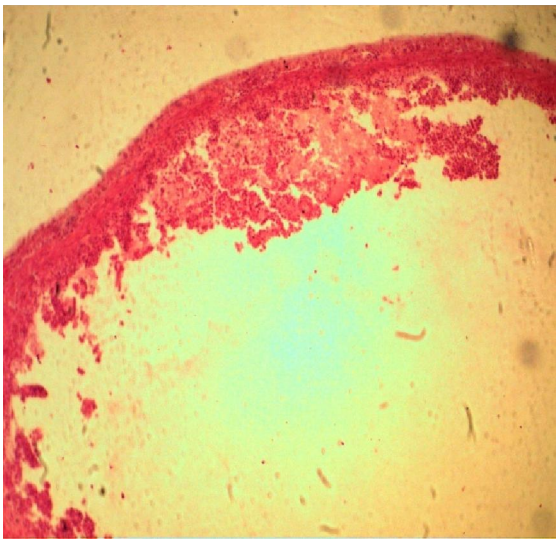

Fig (3): Intestine showing excessive Mucous secreation in intestine .

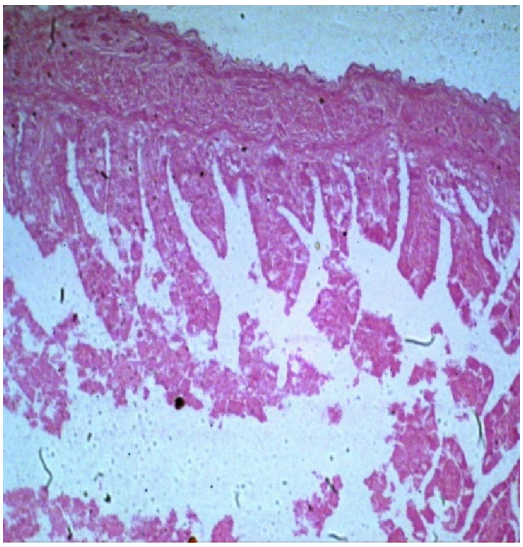

Fig (4): Intestine showing hyperplasia in intestinal epithelium with slight congestion of blood vessels

The embryonated chicken eggs treated with *Aeromonas species (A.hydrophila, A.Caviae, and A.Sobria)* either by inoculation via yolk sac route or dipping were greatly affected. In group A which inoculated via yolk sac route the embryo mortality was 100% (3 days post inoculation) while in group B which dipped in Aeromonas species the mortality rate ranged from 25%, 33.4% and 17% (11 days post dipping) for *Aeromonas A.hydrophila, A.caviae and A.sobria*, respectively.

Reisolation of Aeromonas from liver of dead embryos of group A inoculated by yolk sac route were 83%, 75% and 50% while from yolk results were 91.6%, 75%, 66.6% for *A. hydrophila, A.caviae and A.sobria,* respectively as shown in Table (3).

Group B which had dipped in media containing Aermonas spp. showed 33.3%, 25% and 0% for isolation of *A. hydrophila, A. caviae, A.sobria* from liver while isolation from yolk were 100%, 50, 100% for *A. hydrophila, A.caviae and A.sobria* respectively.

The hatchability of the inoculated ECE was 0%in group A while it ranged from 66.6 – 83% in group B *A. hydrophila* was reisolated from 44.4%, 66.6% from liver and yolk of hatched chicks respectively, *A. caviae* was reisolated from 50 and 62.6 from liver and yolk of hatched chicks. And *A. sobria* was reisolated from 10% and 30% of liver and yolk of hatched chick.

Histopathological examination of liver, intestine of hatched chicks revealed that changes in liver in form of sever congestion in the hepatic blood vessels and sinosoids they were distended and encorged with blood. There was focal areas of coagulative necrosis with disturbance in the arrangement of hepatocytes. The intestine, there was excessive Mucous secreation in intestine as a result of hyperactivity of mucous gland, and sever hyperplasia in the intestinal epithelium with slight congestion of blood vessels in the mucosa. Fig. (1-4).

Discussion

Aeromonas species has public heath significance . It's one of most important organisms which cause a food borne illness (Barnhart and Puncorbo 1992). *Aeromonas hydrophila* cause localized and systemic infections in avian species (Glunder and Siegmann 1989, and Shane and Gifford 1985).

This study aimed to study the effect of *Aeromanas* species: *(A.hydrophila, A.caviae, A.sobria)* on the viability and hatchability of embryonated chicken eggs as well as its pathological effect on hatched chicks.

Results showed that, *A. hydrophila, A.caviae and A.Sobria,* kill the chicken embryos. There was a correlation between the route of infection and mortality rate. inoculation via Yolk sac revealed mortality 100% of the embryos chicken eggs within 3 days post inoculation in group A. . The dipping of ECE in media containing(*A.hydrophila, A.caviae and A.sobria)* revealed mortalities 25%, 33.4% and 17% respectively after 11days post dipping In group B(Tables 1 and 2).

Aeromonas species were reisolated from liver and yolk of dead embryos in both groups A were 83%, 75% and 50% and from yolk were 91.6, 75, 66.6 for *A.hydrophila, A.caviae and A.sobria* respectively.

In group B which dipped in Aeromonas suspension the isolation percentage from liver were 33.3, 25 and 0 while from yolk were 100%, 50% and

100% for *A.hydrophila, A.Caviae and A.sobria*, receptivity.

The differences in mortality percentage and reisolation percentage from liver and yolk of dead embryos could be attributed to the method of infection with Aeromenas . It was found that the yolk sac inoculation route was more effective than the dipping method. Also, the reisolation the of Aeromonas from liver and yolk of group A which inoculated with yolk sac route were higher than group B which dipped in the Aeromonas species. These results aggreeded with the finding abtaned by (Kutkat et al., 2001) as they found that *A. hydrophila* can cause mortality in embryos ranged from 10-20% (after 6 days post inoculation) there was a correlation between the level of *A. hydraphila* and the mortality.

As the inoculation rate results in insert of high level of Aeromonas Inside the ECE & result in 100% mortality while dipping of ECE in the *Aeromonas* suspension results of eggs. From the previous results we can expect the effect of Aeromonas on ECE from infected females may be due to contamination of egg shell by dropping will be less than infection from the ovary.

Aeromonas speices effected on the percentage of hatchability of ECE as shown on Table (1 , 2) the inoculation of *Aeromanas* in the ECE cause mortality of 100% of the so the hatchability percentage was 0% while in the group B which dipped in Aeromonas suspension should hatchability percentages 75, 66 and 83 in *A. hydrophila A.caviae and A.sobria* respectively and these were no significant difference in between different group in hatchability.

The reduction in the hatchability mainly due to mortality of the weak embryos these results agreed with that abtained by (Yadav and Verma, 1998).

Reisolation of the Aeromonas from liver and yolk of the hatched chicks was 44.4% and 66.6% for A. hydrophila 50% and 62% in *A. caviae* and 10 and 30% in *A. sobria*. There was no difference in reisolation percentage from internal organs between A. hydrophila and A.caviae but there were high difference in reisolation percentage from liver and yolk in group dipped in *A. sobria*, in the other hand; the reisolation rate was lower then the other two groups these may be explained by the lower invasion of the of Aeromonas species to the embryo tissues.

Aeromonas strains may be produce Toxins which cause pathological changes in the liver and intestine of the hatched chicks, the liver change were in form of focal areas of coagulative necrosis with disturbance in the arrangement of hepatocytes. In intestine there was excessive mucous secretion and sloughing of the epithelium tissues of intestine with destructed villi.

Results of the present study indicated that, Aeromonas strains A.*hydrophila, A.caviae, A.sobria* were highly pathogenic for chicken and causing embryo mortalities and decrease of the hatchability.

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12/12/2010

Perceptions of Forestry and Rangeland Department Specialists on the Role of Extension-Education Activities to Protect Forests (Case of Mazandaran Province, Iran)

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Abstract: The purpose of this study was to determine the perceptions of forestry and rangeland department specialists in Sari Township regarding the role of extension and education in protection of forests. The population of this study included 230 forest specialists in Sari Township. A stratified random sample of 140 specialists was selected. The research design used for this study utilized descriptive survey research methodology. A questionnaire was developed to assess role of extension-education programs in protection of forests. Findings revealed that education of youth and children at the elementary and secondary school level is also necessary and important in forest protection and conservation. Also results showed that inform to public about worth of forest is very important in protection of forest. The study showed that extension-education methods are important factors in protection of forests.
[Mohammad Abedi, Mohammad Sadegh Allahyari and Khodamoradi. Perceptions of Forestry and Rangeland Department Specialists on the Role of Extension-Education Activities to Protect Forests (Case of Mazandaran Province, Iran). Life Science Journal. 2011;8(1):408-411] (ISSN: 1097 – 8135). http://www.lifesciencesite.com.

Keywords: Forest, rangeland, perception, protection, extension method

1. Introduction

There are an estimated 3870 million hectares of forest worldwide, of which almost 95 percent are natural forest and 5 percent are forest plantations. Also, forest cover about 30 percent of the earth's land area (FAO, 2001). Developed countries for years to come to the conclusion that the destruction of forest trees will exacerbate air pollution, so cutting old trees and destroying forests in these countries is prohibited. However, other countries that still believe in the importance of forests have not lost to the massive destruction of natural resources (Khabbaz, 2010). Tropical deforestation and degradation of forests in many parts of the world are negatively affecting the availability of forest goods and services. While forest area in developed countries has stabilized and is slightly increasing overall, deforestation has continued in developing countries. The estimated net annual change in forest area worldwide during the past decade was –9.4 million hectares; representing the difference between the estimated annual rates of deforestation of 14.6 million hectares and the estimated annual rate of forest area increase of 5.2 million hectares(FAO, 2001). The causes of forest degradation are varied. Some, such as overexploitation of forest products, can be avoided or minimized by sound forest planning and management, whereas the effects of others, such as natural disasters, can be mitigated by contingency planning. Factors responsible for this loss are the conversion of forestland to produce food for a burgeoning world population, especially in developing countries (FAO, 2000; Swanson, 1997), as well as logging for timber and fuel. These are legitimate human needs and uses of forestland. But, lack of knowledge, and legal and social systems often encourage excessive, non-sustainable land clearing resulting in long-term adverse social and environmental impacts (Jones, 1997).

The forestry situation in Iran is no different than other vulnerable areas in the world. There are an estimated 12 million hectares of forest in Iran, while there are 18 million hectares forests in 1950. At present only %11 of this forests are commercial. Figures show that in one year decrease about 12245 hectares forest in Iran (Anonymous, 2009).This forest threatened by unsound forest management activities including inappropriate productivity (too much) by government companies private sector and cooperative, intensive agricultural operations, indiscriminate forest activities and timber use, Lack of vehicles for foresters, smuggling of wood, Lack of near cooperation between forest sector with judicial and disciplinary power, Lack of adequate protection personnel, changing forestlands to agricultural fields, presence of livestock in forests, continuous changes in policies, legislation and programs, lack of education level among personnel, threat of pests and

diseases to plantations, making roads inside forests, lack of participation by forest dwellers in protection of forests, lack of politicians serious belief on the protection of forest, cutting trees by forest dwellers, changing forestlands to agricultural fields, happening of fire and other factors (Anonymous, 2010; Khosrowshahi and Ghavamie, 2008; Farhadian, 2000; Abedi, 2004).

The Forest and Rangeland Organization (FRO) of Iran and its Research Institute are responsible for the management of forests. An office of extension and training was established in the FRO in 1990 to educate and work with these managers and with target audiences of forest landowners and forest dwellers in supporting and participating in forestry. Today, the practice of Extension to improve the management of private and community woodlands is on the increase worldwide (Johnson et al., 2007). Some of the activities including publication of story book in relation to protection of forests for children, the public fair in relation to importance of forests, installing educational poster in the public places, giving presents to students in natural resource week, supplying educational posters in relation to protection of forests and deforestation of forests, organizing educational courses for forest dwellers in relation to preventing and comparing with fire, implementation of theatre in relation to protection of forests and other activities (Abedi, 2004; Farhadian, 2000). Farhadian (2000) studied the FRO's mission and recommended that a strong linkage should be forget between the Office of Extension and Training and the Research Institute. He emphasized that a key responsibility of managers and stuff of the FRO was providing for the participation of people of the planning and implementation of forestry development. According FAO (2000), most forests in the developing world are on land on which indigenous groups and rural communities depend for their livelihood. Therefore, it is essential that they be involved in forest management programs. In a similar vein, Sharma (1999) emphasized that attitude of people influence how they manage and use forests. In a Report of the Islamic Republic of Iran on Forestry Development and key events presented to the twelfth session of the Near East Forestry Commission, it was stated that while forests in different regions of the country important, those of the Caspian Sea Region (Mazandaran and Guilan provinces) are the only economically productive forests in Iran. Mazandaran provinces include Sari and Noshahr Township. Sari Township has important economic role that produces 50% forest products of Iran. There are an estimated 643793 hectares of forest in Sari Township and there are 1186145 forest dwellers and 1628700 livestock in this region. Considering this situation, a study of Forestry and Rangeland Department specialists in Sari Township was considered worthwhile.

The purpose of this study was to determine the perceptions of Forestry and Rangeland Department specialists in Sari Township regarding the role of extension-education in protection of forests.

2. Material and Methods

The research design used for this study was a descriptive survey research methodology. The population of this study included 230 forest specialists in Sari Township. A stratified random sample of 140 specialists was selected. A questionnaire was developed to assess extension-education methods that impact on the protection of forests. 5-point likert-type scale was used to assess expert's self-perceived perception. Content and face validity was determined by faculty and graduate students in the Department of Agricultural Extension and Education at Tarbiat Modares University, Iran. The instrument was pilot tested with 10 forestry specialists in the Forestry and Rangeland Organization under Ministry of Agriculture (Jihad-e-Keshavarzi) two weeks prior to the study, and needed modifications were made. Cronbach's alpha reliability coefficients for sections 1-3 of the instrument ranged 0.72 to 0.93. Data collected were analyzed using the Statistical Package for the social sciences (SPSS, 14). Appropriate descriptive statistics such as mean scores and standard deviations were used to analyze the data generated.

3. Results

According to table 1, the ages of the respondents ranged from 27 to 59.The mean age was 59. Majority (51.4%, n =72) of respondent were 41-50 years old. The mean years served as forest and rangeland experts were 15.5. Majority (53.3%, n =75) of respondent had 11-20 years of experience. Nearly 22.1% of experts had served in Department of Forestry and Rangeland upper than 21 years. 89.2% of specialists had a bachelor's degree and upper and only 10.7.9% of specialists had a technical degree (n= 15).

Table 1. Personal characteristics

characteristics	frequency	percentage
Age		
>30	8	5.7
31-40	39	27.9
41-50	72	51.4
51<	21	15
Level of education		
Technician	10	10.7
Bachelor	108	108

Graduate	17	17
Field of study		
Forestry	72	52.9
Related discipline to rangeland	30	22.1
Agriculture	15	11
Non-related (others)	19	14
Years of experience		
>10	34	24.3
11-20	75	53.6
21>	31	22.1

Table 2 shows the rank important of 38 extension-education methods that effect on protection of forests as perceived by specialists. The most effective extension-education methods were publication book for children about protection of forest (M=4.582), putting forest subjects in schoolbooks (M=4.478) and writing device on vehicles regarding protection of forests (M=4.464). Organizing educational courses for forest dwellers in relation to preventing and comparing with fire and implementation of camp for students in relation to importance of forests were considered to be the least important factors. Also high rate of mean scores indicated that extension-education methods were very effective in protection of forests.

Table1: extension-education methods that effect on protection of forests as perceived by specialists

Rank	Extension-education methods	Mean	SD
1	Publication of story book in relation to protection of forests for children	4.582	0.61
2	Inserting appropriate subjects in relation to protection of forests in school books	4.478	0.67
3	Writing device on the public vehicles in relation to protection of forests	4.464	0.70
4	Writing sub-title in relation to protection of forests in TV	4.464	0.76
5	presenting video movies in relation to protection of forests for forest dwellers	4.463	0.73
6	Establishing special radio of forest for giving information to protection power	4.463	0.75
7	Existence technical-professional courses in relation to protection of forests	4.400	0.82
8	Educational courses for experts in relation to protection of forests	4.400	0.85
9	The public fair in relation to importance of forests	4.392	0.81
10	Presenting program in relation to protection of forests in before and after news of TV	4.385	0.81
11	Writing device on the calendars and books in relation to protection of forests	4.355	0.87
12	Distribution of publication for public in relation to protection of forests	4.347	0.85
13	Sending experts to other countries for observation forestry methods	4.321	1.01
14	Impression of stamp in relation to protection of forests	4.271	1.10
15	Writing device on schools educational tableau	4.270	0.90
16	Diffusion successful patterns in relation to public participation	4.262	0.89
17	Installing educational poster in the public places	4.251	0.90
18	Giving presents to students in natural resource week	4.221	1.01
19	Implementation of educational courses for forest dwellers	4.085	0.97
20	Writing device in relation to protection of forests in administrative letters elderhand	4.078	0.98
21	Organizing educational courses for forest dwellers in relation to comparing with fire	4.035	0.90
22	Implementation of competition for forest dwellers	3.964	1.10
23	field trip for forest dwellers	3.907	1.13
24	Supplying educational posters in relation to protection of forests	3.900	1.07
25	Ringing schools bell due to natural resource week	3.899	1.05
26	Radio educational programs in relation to protection of forests	3.821	1.14
27	Publication subject in relation to protection of forests in newsletter and magazines	3.764	1.12
28	Giving scholarship for children of forest dwellers	3.735	1.20

29	Implementation of camp for students in relation to importance of forests	3.712	1.13
30	Promoting literacy of forest dwellers	3.650	1.13
31	Publication important individuals speech (scientific, athletic, artistic,...) in relation to importance of forests	3.618	1.13
32	Existing extension agent of forest	3.607	1.07
33	Implementation of theatre in relation to protection of forests	3.550	1.15
34	Public walking due to natural resource week	3.528	1.23
35	Using literacy campaign programs for forest dwellers	3.525	1.11
36	presenting TV movies in relation to protection of forests for public	3.514	1.32
37	implementation of competition(scientific, painting, ...) in relation to protection of forests for public	3.364	1.28
38	Selecting pattern forester	3.300	1.20

Likert-type scale: 1= strongly disagree, 2= disagree, 3= moderate, 4= agree, 5= strongly agree

4. CONCLUSION

A range of extension methods was considered by specialists to be effective in protection of forests. Among this methods, publication of the story book in relation to protection of forest and inserting appropriate subjects in relation to protection of forest in school books was presented as most important methods. Therefore education of youth and children at the elementary and secondary school level is also necessary and important in forest protection and conservation. Also conclusions showed that inform to public about worth of forest is very important in protection of forest.

The study showed that extension-education methods are important factors in protection of forests in comparing with other factors. Therefore, there is a necessity that we work on the extension-education methods in relation with protection of forest in various countries proportion with diverse cultures.

17/01/2011

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Dental Caries Prevalence among a group of Egyptian Nurseries Children

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Abstract: Dental caries in children attending nurseries has a significant dental public health problem and health disparity implications. **Aim**: This study was carried out to investigate the prevalence of dental caries among a group of Egyptian children at nurseries in El Kalubia Governorate, Egypt. **Subjects and Methods**: A total number of 999 Egyptian children 496 boys and 503 girls with their ages ranged from three years to less than six years. Children were selected randomly and examined from those attending nurseries. The examined children were subdivided according to their ages into five age groups with six month intervals. Clinical examination was conducted at the nurseries and was exclusively visual with the help of tongue depressor according to the World Health Organization oral examination criteria for mass population and carried out under natural day light with children lying on ordinary desks. **Results**: Dental caries prevalence was high among the study subjects (60.4%) with the mean dmf value 3.31±3.99. The results of this study showed that the prevalence of dental caries increases with age and there is no statistically significant difference between boys and girls (P value equal 0.24). The sequence of caries attacks follows specific pattern where the right mandibular first primary molar had the highest caries percentage (36.5 %) then maxillary molars and maxillary anteriors teeth and finally the left lower primary lateral incisor which exhibit the lowest caries percentage (1.00%). **Conclusions**: Dental caries prevalence is relatively high among the examined subject and these findings stress the need for implementing an effective oral health preventive program for those children as well as an educational dental health programs for their guardians and teachers to improve their oral health status.

[Abou El-Yazeed, M.; Rashed, M.; El sayed, M.; Salah, A. **Dental Caries Prevalence among a group of Egyptian Nurseries Children.** Life Science Journal. 2011;8(1):412-419] (ISSN:1097–8135). http://www.lifesciencesite.com.

Keywords: Dental Caries; prevalence; El Kalubia Governorate.

1. Introduction

Dental caries is one of the most prevalent chronic diseases in mankind worldwide. It is a multifactorial disease that starts with microbiological shifts within the complex biofilm (dental plaque). Caries is affected by the consumption of dietary sugars, salivary flow, exposure to fluoride and preventive behaviors, it is therefore very important to prevent dental caries, but this will not be successful unless the available scientific knowledge about changing the etiological factors of the disease is applied (Poureslami and Amerongen, 2009).

Dental caries is an infectious, transmissible bacterial disease; the most predominant bacterial species are streptococcus mutans and lactobacilli species, as in ordinary pattern of dental caries (Selwitz et al., 2007). In children the caries attack pattern depends on three factors: the timing of the tooth eruption, the time span of the harmful dietary habit and the type of muscle movements during sucking and swallowing. Many authors reported that the attack pattern of the early childhood caries changes at age three, when it begins to affect the first and second primary molars (Brodeur et al., 2006; Hallett et al., 2006 , Berkowitz ,2006 and Andrea, et al 2010.

A comprehensive review of the epidemiology of dental caries in children showed that its prevalence varies from population to another (Kaste et al., 1992, Milnes, 1996 and Wyne, 1999 and Leake et al., 2008). The World Health Organization's 2003 report on oral health provides an overview of global caries epidemiology that confirms its international pandemic distribution. Globally, WHO reports caries prevalence in school-age children at 60–90%. The American Centers for Disease Control and Prevention released report which revealed high ongoing prevalence of dental caries in children, with 27% of nurseries, 42% of school-age children and 91% of adults having caries (Petersen et al., 2005 and Edelstein, 2006). In Boston, United States the prevalence of early childhood caries in 1- to 3-year-old children seeing primary-care pediatricians at two urban medical centers in Boston was compared to that prevalence in similarly aged US children surveyed as part of the Third National Health and Nutrition Examination Survey, it was found that overall the prevalence among one to three year-old urban Boston children was 3.0 %, while the

overall prevalence among one to three years old US children was 6.3 % (Nunn et al., 2009). In Brazil a cross sectional study was done to investigate the prevalence of caries in children from 0-36 months and its relationship with sugar consumption, it was found that caries was present in 38% of the babies, and an average dmf-t was 3.208 (Peelm et al., 2008). In Australia a cross-sectional sample of 2515 children aged from 4 to 5 years was examined in nurseries using decayed, missing, filled teeth/surface (dmft/dmfs), 12.3% had an anterior caries pattern, 21.4% had a posterior pattern, 24.3% children had non-severe caries and 29.4% had severe caries experience. The sample mean dmft was 1.4 ± 2.77 and the mean dmfs was 2.28 ± 6 (Hallet and O'Raurke, 2006). In Kai Fu Changsha, China a cross sectional study was conducted to investigate the dental caries prevalence in primary teeth among nurseries children the results showed that the prevalence of dental caries was 39.65% and the mean dmft score was 1.32(Que and Hau, 2009). In Switzerland a cross sectional study was conducted to describe the caries prevalence in two years old children, it was found that about 12.6% of the children had early childhood caries and about 4.4% of the children had severe early childhood caries with the mean dmft was 4.3 (Menghini et al., 2008). In Israel a cross sectional study was undertaken to assess the prevalence of dental caries in the earliest age at which children were organized as a group in the national education system and to find possible associations with variables that may help to identify "groups at risk", in this population a total of 965 children, 5 years old were examined, it was found that 84% of the children were affected with mean dmf of 4.7 $\pm$ 3.6 (Zadik, 2006). In Egypt the prevalence of early childhood caries varied among different researches. A study in Cairo was done to investigate dental caries prevalence in 900 child with their age ranged from 3-12 years old. The mean defs was 10.2 and it reached its maximum level at age 8-9 years old, the mean DMFT reached its maximum level at age 11-12 years (El-Sayed, 1996). Another study in Cairo was carried out to determine the prevalence of early childhood caries among Egyptian children by reviewing the patients assessment charts of the patients attending the Pediatric Dentistry Department, Faculty of Oral and Dental Medicine, Cairo University throughout the year 2003-2004, the results of this study revealed that the prevalence of early childhood caries among the children attending the department clinics was 8.022%, while boys showed higher caries prevalence than girls (Awad, 2006). In Giza governorate a cross sectional study was conducted to investigate the prevalence of nursing caries among 2073 nurseries children from rural areas with their age ranged from 1-4 years, it was found that the prevalence of dental caries was 14.32% with females were more affected more than males (Abd El-monem, 1997).

Aim of the study

Dental caries is considered a major health problem in the field of pediatric dentistry; as its prevention and management require intervention of multidispelnary approaches, from this scope this study was carried out to throw light on the situation of dental caries among a group of Egyptian children at some nurseries in El Kalubia Governorate.

2. Subjects and Methods

The protocol of this study was first submitted to the ethical committee of the National Research Centre, Egypt. A consent form was obtained from the nursery principles to conduct this study, as well as a written permission was also obtained from the parents or guardians to perform the examination. Only children of parents or guardians who gave permission were included in the study. A total number of 999 Egyptian children were included in this study, their ages ranged from three to less than six years old. Children were classified into two main groups (males and females). Each group was subdivided according their ages into five subgroups with six months interval. Subgroup I - Children with an age ranged between (3-3.5), II - Children with an age ranged between (>3.5 -4), III - Children with an age ranged between (>4- 4.5), IV - Children with an age ranged between (>4.5- 5) and V - Children with an age ranged between (>5- <6). Personal data including name, age, sex, address and site of the nursery were recorded. The examination was carried out in the day light, with the children lying on ordinary desks facing a window in the class room. In this study decayed (d), missed (m) and filled (f) primary teeth (dmf) index was used. All data were analyzed using Microsoft Excel Software with statistical subroutines add in SPSS version 11. The obtained data arranged, tabulated and subjected to statistical analysis using non parametric tests for interpretation. Non parametric tests were used to test the level of significance between boys and girls, maxillary and mandibular teeth as well as between both the right and left sides (independently).

3. Results

The dental caries prevalence among the examined subjects was 60.4% with the mean dmf values was 3.31±3.99. Comparison between caries experience in boys and girls shows that the dmf values for girls had higher prevalence as compared to that of boys where the mean dmf equal 3.20±3.91 and 3.41±4.01 for boys and girls respectively but no

statistically significant difference was found between them where P value equal 0.2430 (Table, 1). As regarding the dmf values of the maxillary and mandibular teeth, highly statistically significant difference was noticed when comparing their values where P value equal zero, meanwhile comparing the dmf values of the right and left sides showed that there is no statistically significant difference between them with P value equal 0.5184 (Table, 1).

Table (2) and Figure (1) shows the total mean of dmf values for the five age groups separately, where the mean±SD were 2.5806±3.3729, 2.5067±3.8847, 2.9520±3.9863, 3.3667±3.5992 and 3.7723±4.1323 respectively and a statistically significant difference was found between them where P value equal o.oo5.

Table (1): Comparison between caries experience in boys and girls, maxillary and mandibular teeth as well as right and left sides

Caries experience	Dmf (Mean ± S.D.)	P value
Boys	3.20±3.91	0. 2430
Girls	3.41±4.01	
Maxillary teeth	1.33 ± 2.14	0.000*
Mandibular teeth	1.98 ± 2.34	
Right side	1.64±2.06	0.5184
Left side	1.98±2.34	

*Significance at P < 0.05

Table (2): Mean dmf values for the five age groups

Caries experience	Group I Mean ± SD	Group II Mean ± SD	Group III Mean± SD	Group IV Mean± SD	Group V Mean± SD	P value
dmf	2.5806±3.3729	2.5067±3.8847	2.9520±3.9863	3.3667±3.5992	3.7723±4.1323	0.0052

*Significance at P < 0.05

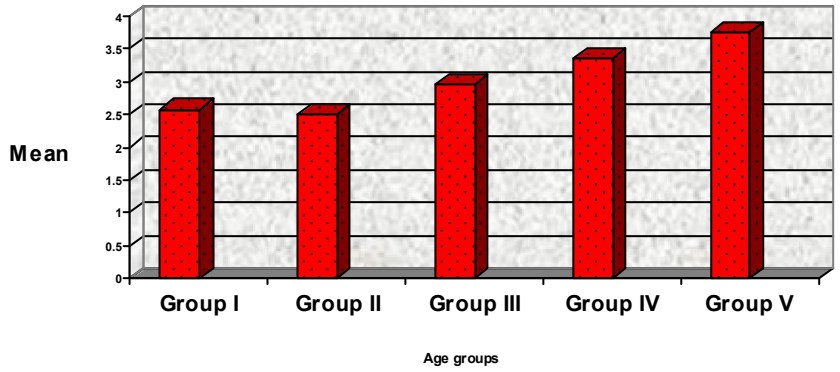

Figure (1): Mean dmf values for the five age groups

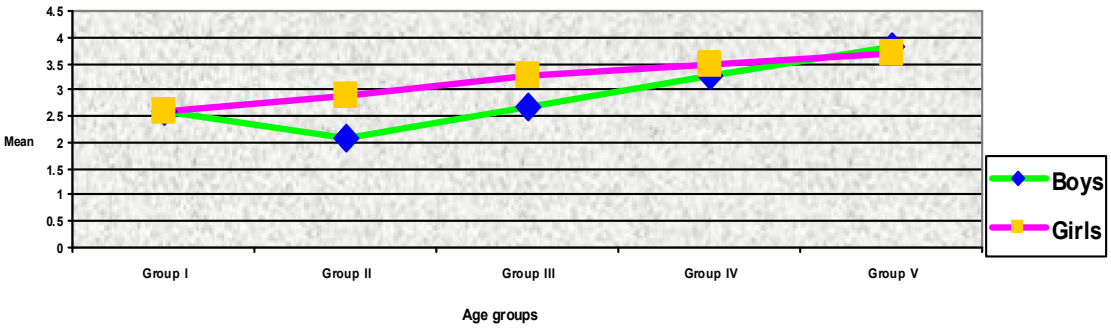

Figure (2) Mean dmf values of caries experience for boys and girls

Table (3) Mean ± Standard Deviation of the dmf values of the five age groups by arch and side

Variables	dmf values														
	Group I Mean ± SD		P value	Group II Mean ± SD		P value	Group III Mean ± SD		P Value	Group IV Mean ± SD		P value	Group V Mean ± SD		P value
	Boys (n=48)	Girls (n=45)		Boys (n=35)	Girls (n=40)		Boys (n=147)	Girls (n=124)		Boys (n=45)	Girls (n=45)		Boys (n=221)	Girls (n=249)	
Right side	1.38 ± 1.82	1.29 ± 1.79	0.82	1.02±1.67	1.40±2.15	0.41	1.26±1.80	1.59±2.18	0.18	1.51±1.82	1.71±1.77	0.60	1.95±2.27	1.85±2.08	0.61
Left side	1.21 ± 1.61	1.29 ± 1.69	0.82	1.05±1.96	1.47±2.02	0.37	1.43± 1.95	1.66±2.29	0.38	1.75±2.12	1.75±1.89	1.00	1.87±2.09	1.86±2.18	0.96
Upper arch	1.14 ± 1.82	1.06 ± 1.85	0.84	1.05 ± 2.32	1.32± 1.95	0.59	1.14± 2..09	1.21 ± 2.15	0.79	1.51 ± 2.30	1.15± 1.09	0.59	1.60 ±2.31	1.35±2.12	0.23
Lower arch	1.44 ± 1.92	1.51 ± 1.94	0.86	1.02±1.58	1.55±2.41	0.27	1.54±2.00	2.04±2.57	0.08	1.75±1.89	2.31±2.18	0.58	2.23±2.37	2.36±2.59	0.56
Total	2.58±3.36	2.58±3.43	0.78	2.09±3.60	2.88±4.13	0.51	2.69±3.63	3.26±4.37	0.13	3.27±3.77	3.47±3.45	0.49	3.83±4.19	3.71±4.09	0.38

* Significance at $p < 0.05$

Table (3) shows the mean ± standard deviation of the dmf values of the five age groups by arch and side. It was observed that boys and girls of age group five have the highest dmf for both right and left sides. By assessing the dmf value of both upper and lower arch it was observed that boys of age group two have the lowest dmf values for both upper and lower arch.

The percentage of decayed, missed and filled primary teeth for all examined children was shown in table 4. It was found that the upper right first primary molar had the highest decayed and filled percentage where d component equal 19.2% while for the filled percentage f component equal 1.1%. On the other hand the upper right primary canine had the lowest decayed and filled percentage where d component equal 2.1% and f component equal zero. As regarding the percentage of decayed, missed and filled lower primary teeth for all examined children. It was found that the lower right first primary molar had the highest decayed percentage where its d component equal 36.5 while the lower left primary lateral incisor had the lowest decayed percentage where its d component equal 1.00. It was also noticed that there were no missing lower primary lateral incisors and lower right primary canine where their m component equal zero.

Table (4): Percentage of decayed (d), missed (m) and filled (f) teeth for all examined children in both arches

Tooth	% d	% m	% f
URE / LRE	16.6 / 30.5	0.30 / 2.00	0.00 / 2.90
URD / LRD	19.2 / 36.5	1.80 / 2.70	1.10 / 2.70

URC / LRC	2.10 / 1.90	0.00 / 0.00	0.00 / 0.00
URB / LRB	6.30 / 1.10	0.20 / 0.00	0.00 / 0.00
URA / LRA	12.1 / 1.30	0.50 / 0.50	0.00 / 0.00
ULA / LLA	11.8 / 1.30	0.50 / 0.20	0.00 / 0.00
ULB / LLB	7.00 / 1.00	0.20 / 0.00	0.00 / 0.00
ULC / LLC	2.50 / 2.30	0.00 / 0.10	0.00 / 0.00
ULD / LLD	18.8 / 34.3	2.00 / 3.30	0.90 / 3.00
ULE / LLE	16.5 / 30.2	0.20 / 2.00	0.60 / 3.40

4. Discussions

Information about dental caries prevalence and severity forms the basis for caries prevention programs and treatment needs in population; therefore there is a continuous need for more studies about the prevalence and severity of dental caries. The present study was carried out to determine the caries prevalence among a group of Egyptian nurseries children in El Kalubia Governorate. In this study the mean dmf of the total subjects shows lower values than the results of similar studies that were conducted in Kuwait by Vigild et al., 1996 and in Saudi Arabia by Wyne, 2008, this may be attributed to the difference in the life style and socioeconomic level of the children of these countries as they consume more cariogenic food, junk food and drink high amount of carbonated soft drinks as compared to that of Egyptian children.

On the contrary it was found that the mean dmf of the total sample was higher than the results of Masiga and Holt, 1993 in Nirobi and a similar study conducted on Brazilian nurseries children aged from 2-6 years done by Freire et al., 1996, this may be attributed to the low socioeconomic status of the children in these countries which in turn may lead to the decrease in consumption of sweets and carbonated soft drinks. It was observed that the dmf values increase as age increases. As age factor is considered in all articles the most important factor that affects dental caries prevalence, so with age increase the higher will be the dental caries prevalence (Que and Hou, 2009), this may be related to the fact that as the age increases the time that the teeth subjected to cariogenic food will also increase which may raise the possibility for decay, these observations were confirmed by the results of similar studies conducted by Allukian, 2000, Saravanan et al., 2008 and Que and Hou, 2009. Although there was gradual increase in the dmf values by age along the five examined groups no statistical significant differences were found between them, this may be attributed to the short age interval between groups. On the other hand when correlating the dmf values between group one and five a statistically significant difference was found, this is in agreement with the results obtained by Ferreira et al., 2007 and Simin, 2010 and may be related to the increase of age interval (three years) between group one and five. The comparison between the dmf values of the upper and lower teeth showed a significant relation, this may be due to the morphological variation of the occlusal anatomy as well as variation in the chronology between the upper and lower primary teeth; this is in agreement with the work of Al Hadded et al., 2006.

There was no statistically significant difference between the dmf values of the right and left sides, formally we can explain this by the absence of morphological or chronological difference between them, as both the right and left primary teeth nearly erupt around the same average eruption dates (Mc Donald et al., 2005). The present study showed no statistical significant difference by comparing the male and female dmf values where P equal 0.2430

which is in agreement with varying studies in other countries (Segovia-Villanuve et al., 2006 and Wanjau and Plessis 2006), this may be attributed to the fact that the dietary and oral hygiene practices related to dental caries are mostly controlled by parents/guardians at this early age, consequently it is considered too early to develop any gender difference in caries prevalence between males and females at this age.

Concerning the caries prevalence on the scale of tooth unit for the entire study sample, it was found that among the anterior primary teeth, the maxillary primary central incisors
were the teeth with the highest caries prevalence where the decayed percentage for upper right primary central incisors equal 12.1% and for upper left primary central incisors equal 11.8%. Our results are similar to a study conducted on Tanzanian and Saudi nurseries children applied by Kerusno et al, 1991 and Wyne et al., 2002 , this is due to the close interproximal contact between maxillary central incisors and the direct exposure during intake and pooling of cariogenic fluid around these teeth. On the other hand the lower primary incisors had the lowest decayed percentage among the anterior teeth this is explained by to the fact that the lower incisors are protected from direct exposure to acidic food by the tongue, also they are close to the sublingual salivary gland duct where it helps in diluting the acidic environment around the lower incisors, this result was confirmed by the results recorded by Wyne et al., 2001 and Carino et al., 2003.

Among the posterior teeth it was found that the mandibular first primary molars showed the highest caries prevalence where the decayed percentage was 34.3% and 36.5% for the right and left primary molars respectively, these results are similar to the work of Kerusno and Honkala, 1991. The increase of the caries prevalence in the first primary molar may be attributed to the tooth morphology as well as to the fact that it is the first erupting primary molar in the mouth. Meanwhile the upper second primary molars had the lowest decayed percentage among the posterior teeth, this may be attributed to their closer proximity to the parotid gland duct and their eruption chronology as it is the last primary teeth erupt in the mouth (Mc Donald et al., 2005).

Analysis of each component of the total dmf index of the sample showed a marked influence of the decayed component upon the total values of dmf scores, this is in agreement with Wei et al., 1993, Mattos-Graner et al., 1996 and Shang et al., 2008. The high decayed component of dmf score indicates huge unmet treatment need among this young population, this is may be attributed to the lack of community awareness about the idea that prevention and treatment of caries should begin at early childhood, also to the bad cultural beliefs which assume that primary teeth should not be treated as it will be replaced by permanent teeth, these beliefs may influence dental caries prevalence especially in disadvantaged communities.

5. Conclusion

Dental caries prevalence is high among nurseries children in El Kalubia Governorate, Egypt (60.4%) with the mean dmf value, 3.31+ 3.99. No statistical significant difference was found neither between dmf values of boys and girls nor between values of right and left sides, meanwhile this difference was significant between upper and lower teeth. The untreated decayed teeth dominated the dmf score among all examined children. This study documents widespread neglect of oral health of nurseries children at El Kalubia Governorate, Egypt.

Recommendations

1. Dental health education programs must be carried out for the children, their parents and their nursery care takers through the collaboration of all community organizations.
2. It is necessary to develop broad national strategy for the implementation of dental caries preventive measures.

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12/11/2010

A retrospective study: The Influence of human immunodeficiency virus co-infection with hepatitis C virus or hepatitis B virus on the efficacy with HAART in China AIDS area

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Abstract: To evaluate the impact of human immunodeficiency virus (HIV) co-infection with hepatitis C virus (HCV) or hepatitis B virus (HBV) on the efficacy of highly active anti-retroviral therapy (HARRT) and analysis on the variable pattern of resistant's sites in HIV RNA.The patients were divided into three groups: HIV/HBV/HCV co-infection group (23 patients), HIV/HCV co-infection group (168 patients), and HIV-only group (178 patients). All patients in the 3 groups were given the same HAART, that was, AZT+DDI+NVP, but not given other antivirus treatment including HCV and HBV antivirus therapy. HIV RNA, HCV RNA or HBV DNA were detected by real time PCR every 90 days, meanwhile the counts of CD_4^+ T lymphocyte and liver function including ALT(alanine transaminase), AST (aspartate aminotransferase), and total bilirubin (T-Bil) were tested. According to the titer of HIV RNA(>10^4copies/ml) in sera during the one year HAART, polymerase genes of HIV RNA were sequenced and analyzed. During one-year HAART, HIV RNA of HIV-only group, HIV/HBV/HCV co-infection group and HIV/HCV co-infection group decreased significantly from 6.78±1.08, 6.23±1.34, 6.54±1.23 log copies/ml to 0.53±0.15, 0.67±0.16, 0.43±0.11 log copies/ml respectively (*P*-Value < 0.001). And CD_4^+ T lymphocyte counts of the three groups elevated significantly from 197±127, 184±113, 213±143 cells/μl to 382±74, 383±70, 378±76 cells/μl respectively (P-value<0.001). However there were no differences among the three groups in HIV RNA and CD_4^+ T cell counts. There were no differences in liver functions including ALT, AST and T-Bil among the three groups. The detection of sites of drug resistance: the major mutant sites to AZT+DDI were at M41L, E44A, K70KR, D67N, L210W, T215Y or K219W which were highly resistant and to NVP were at A98G, V179H, Y181C,K103N or G190A which were highly resistant in the 3 groups. Meanwhile, the rates resistant of emergence were similar and there were no sites to 3TC and protease inhibitors (PIs) in the above HAART groups. HIV co-infected with HBV and/or HCV does not impact on the efficacy of HAART. What more, HAART does not impact HCV replication.
[ZHAO Jie, YU Zu-jiang, KAN Quan-cheng, LI Xiao-fei, LI Zhi-qin, LIANG Hong-xia. **A retrospective study: The Influence of human immunodeficiency virus co-infection with hepatitis C virus or hepatitis B virus on the efficacy with HAART in China AIDS area.** Life Science Journal. 2011;8(1):420-424] (ISSN:1097-8135).

Key words: HIV; Co-infection; High active anti-retroviral therapy

1. INTRODUCTION

HIV, HBV and HCV share similar routes of transmission, with sexual, parenteral and perinatal transmission being the most frequent modes of acquiring these infections. In contrast, exposure to these viruses is followed by an immune response which differs markedly in its ability to clear the infection[1]。Highly active antiretroviral therapy (HAART) has improved the life expectancy of HIV infected patients, but, by extending survival, it permits the development of HCV cirrhosis. Relatively little is known regarding hepatitis viral co-infections among HIV infected patients, so this study was therefore carried out to estimate the effects of HBV and/or HCV seropositivity in a cohort of people living with HIV/AIDS in China and to investigate the effect to these viruses on $CD4^+$ lymphocytes in the HAART.

2. MATERIAL AND METHODS

(1) HIV RNA, HBV DNA and HCV RNA ELISA kits were from Invitrogen Co., Netherlands. Extractive kits for PCR product were obtained from QIAGEN Company (Germany). Real- time PCR was from Roche Co. Sephadex G-100 and Sepharose CL-4B were from Pharmacia Ltd. FACS was from Beckman (USA).

(2) Blood was collected aseptically into 10 ml vacutainer tubes (BD, NJ USA) for biochemical, CD4+ count and viral serology tests. Biochemical and CD_4^+ T lymphocyte assays(FACS, Beckman, USA), were performed within three hours of collection, while serum for serological assays of hepatitis B and C markers were stored at -20°C until

the time for assay.

(3) Extraction of HCV RNA, HIV RNA or HBV DNA was according to the extractive kit's direction. Brief to say, the extracted HIV RNA from the 100μl serum was amplified by PCR with reverse-transcriptase procedure 42℃,45min and 94℃,5min, and so rounded to major procedure:92℃ 30 s, 55℃ 30s,72℃ 30 s for 45 cycles. Amplification products were resolved by agarose gel electrophoresis, stained with ethidium bromide. If HIV RNA was positive (>10^4copies/ml) from the patients who had been given 3 months' treatment of HAART, The polymerase gene of HIV RNA was amplified and sequenced. The resistant sites were analyzed by resistant soft and resistant data provided from Stanford University. The serum amino-transferase determined was ALT, AST, and total T-Bil. The catalytic activity of ALT, AST and T-Bil was determined in serum using a COBAS MIRA chemistry analyzer (GMI, MI, USA) after it was calibrated.

(4) Continuous variables were expressed as mean±standard deviation and were compared using the Mann-Whitney test or the student-t test. Categorical variables were expressed as proportions, compared using the chi-square test or Fisher exact test to evaluate differences between proportions. Datas were analyzed with SPSS (Statistical Package for Social Sciences) program version 10.0; $p<0.05$ were considered statistically significant.

(5) Selection of patients: One hundred and ninety-one naïve HIV/AIDS patients were selected from confirmed HIV-1 positive from China AIDS area. HIV, HBV and HCV infection would be testified by ELISA and western-Blot. Of the 191 patients, the sero-prevalence of HIV/hepatitis viruses were as follows: HIV only was 178 patients; HBV/HIV only 2(This type of patients was too rare and was not statistical analysis), HCV/HIV 166, and HIV/HBV/HCV were only 23 patients。

(6) Regimen of HAART: HIV-1 infected patients on failing HAART were prospectively submitted for consultation all patients were given the same treatment, that was AZT+DDI+NVP (AZT, zidovudine, 600mg/day; DDI, dideoxyinosine, 400mg/day; NVP, nevirapine, 400mg/day, for one year, but not given anti-HBV or HCV therapy corresponding.

(7) Periods of assay: HIV RNA, HCV RNA and HBV DNA in sera were assayed by real-time PCR. Resistance tests, antiretroviral history, adherence, CD_4^+ counts, HIV-RNA levels and HCV/HBV co-infection were scheduled every 90 days during the HAART.

3. RESULTS

(1) Counts of CD_4^+T lymphocyte: After giving HAART for 1 year, there were obviously significant in the comparison of CD_4^+T lymphocyte counts and those were all elevated in every group, that is HIV/HCV/HBV, HIV/HCV and HIV group （P<0.001） (see Table 1). But there were no differences in the **CD_4** counts among the above 3 groups（P>0.05）.

Table 1. **CD_4^+**T lymphocyte Counts in AIDS pre and post HAART（/μl $\bar{x}$ ±s）

Group	No.	Pre- HAART	Post-HAART	P value
HIV/HCV/HBV	23	184±113	378±76	0.001
HIV/HCV	168	213±143	383±70	0.001
HIV	178	197±127	382±74	0.001

(2) Real-time PCR

π. HIV RNA: In the AIDS serum of all the 3 groups, levels of HIV RNA were continuously declined during HAART and there were statistically significant in the copies of HIV RNA between the pre and post HAART in each group (see table 2). On the otherwise, the ranges of decreasing of HIV RNA were the similar and there were no differences among the 3 groups during the HAART（P >0.05, see figure 1）.

Table 2 **HIV RNA in the sera pre and post HAART**（log copies/ml，x±s）

Group	No.	Pre- HAART	post-HAART	P value
HIV/HCV/HBV	23	6.23±1.34	0.67±0.16	0.001
HIV/HCV	168	6.54±1.23	0.43±0.11	0.001
HIV	178	6.78±1.08	0.53±0.15	0.001

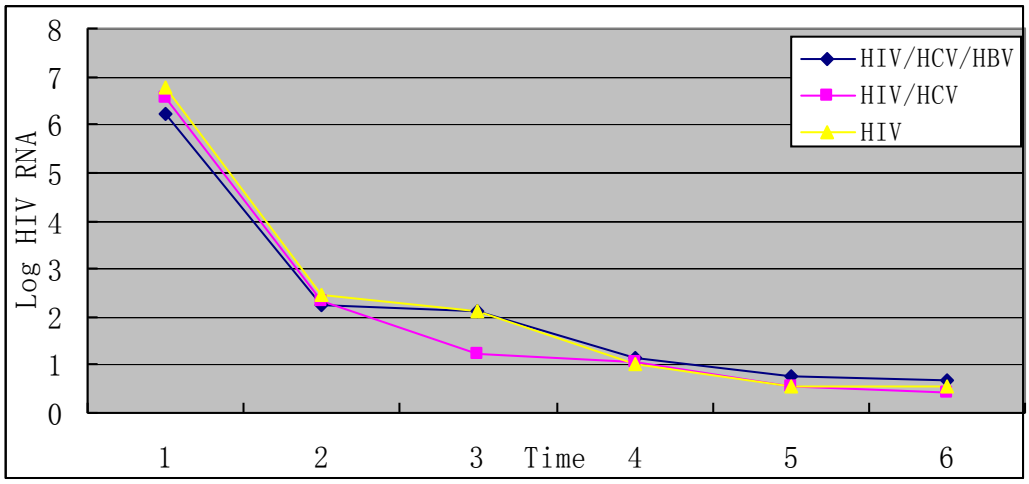

Figure 1 HIV RNA in the sera during the HAART by real-time PCR assay（$x\pm s$）

θ. HCV RNA: Of the 23 patients with HIV/HCV/HBV co-infection, HCV RNA position is 21 and 163 cases with HCV RNA(+) in all 168 HIV/HCV patients. From HIV/HCV/HBV or HIV/HCV co-infection groups, level of HCV RNA had no overt alternation and was often in the range about 10^7 ~10^8 copies/ml during the HAART(see table 3). But to HIV RNA, it was obviously declined in the HIV/HCV co-infection group. Statistic data showed that it was no significant in HCV RNA between pre and post HAART **(**see figure 2).

Table 3. HCV RNA in sera at pre or post HAART（log copies/ml，$x\pm s$）

Group	No.	Pre-HAART	Post- HAART	*P* value
HIV/HCV/HBV	21	7.23±1.54	6.86±1.36	0.67
HIV/HCV	163	6.76±1.47	7.15±1.41	0.64

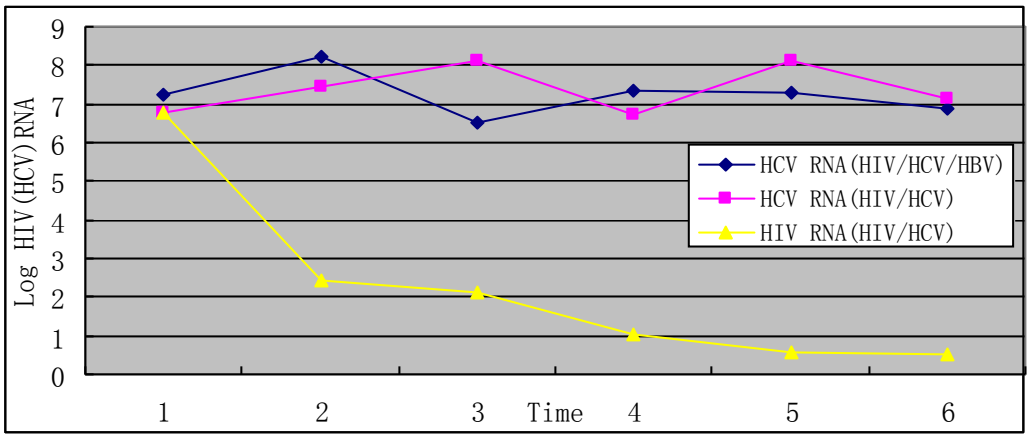

Figure 2. HIV RNA or HCV RNA in the sera during the HAART（$x\pm s$）

ρ HBV DNA: During the HAART, the change of HBV DNA was the similar as the trend of HCV RNA, but it was in low level about 10^3~10^5 copies/ml. Statistic showed that it was no significant in the level of HBV DNA between pre and post- HAART.

σ Liver function: When clinical features and biochemical markers were considered together, ALT in only 1 patient with any of the markers for hepatitis had elevated (by history or examination). But in the following treatment, it became normal after 1 month. Importantly, only 5.1% of the patients co-infected with hepatitis B or C virus co-infection had abnormal serum ALT or AST.

τAssay of resistance: The resistant strains were emergence since the third month with HAART, but only 7.8% of the patients co-infected with hepatitis C virus co-infection had abnormal serum HIV RNA in all groups. The

rates and positions of variant sites were the similar as the other 2 group patients. The sites for NRTI (AZT and DDI) were major in M41L, E44A, K70KR, D67N, L210W, T215Y, K219W A98G, V179H, Y181C, K103N, G190A, K20R, V35L, K43E, W88C, K122E, I135V, S162C, G196E, T200A, E203D, H221Y etc. And for NNRTIs(NVP), there was only in Y181C (see figure 3).There were no resistant sites to 3TC and protease inhibitors (PIs) in the above HAART groups.

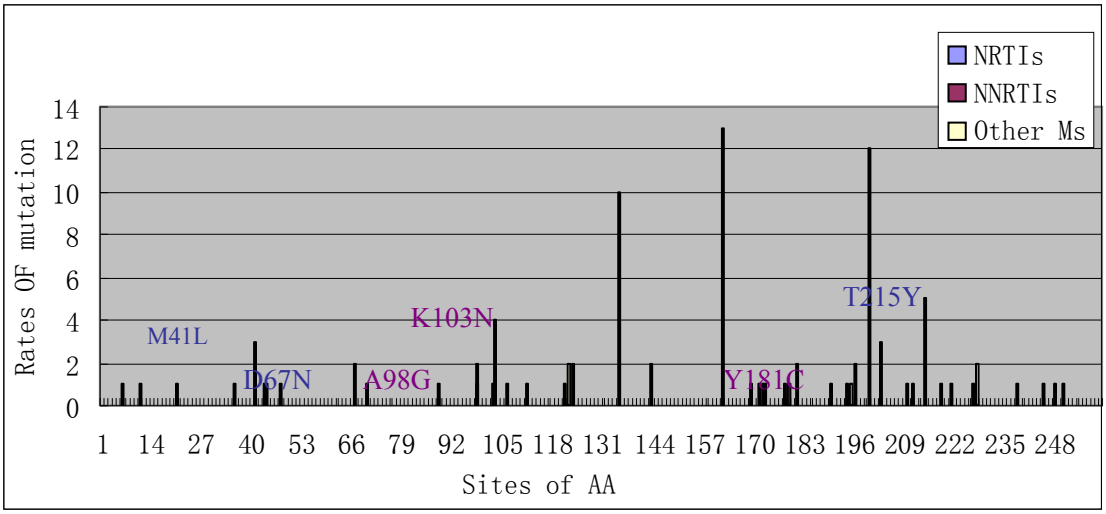

Figure 3. Rate and position in the resistant strain of polymerase gene in the patients co-infected with hepatitis virus C.

4. DISCUSSION

HIV, HBV, and HCV are devastating disease agents that share common modes of transmission (Vincent Soriano, Pablo Barreiro and Marina Nuñez), therefore HIV positive individuals are at risk of co-infection with HBV and HCV infections[2]. With the increased lifespan of HIV-1 infected patients, HCV and HBV have recently emerged as important pathogens in these patients[3]. With the advent of highly active antiretroviral therapy (HAART) regimens capable of dramatically prolonging the survival of HIV-infected patients, the impact of co-morbid infections such as HBV and HCV has come into focus. Co-infection with HBV or HCV increases the risk for hepatotoxicity of HAART and likelihood of onset of an AIDS-defining illness, compared with infection with HIV-1 alone. Although the HIV co-infection with HBV and/or HCV has been recognized worldwide, limited data are available on the extent of co-infection. Few studies have been done on HIV, HBV, and HCV separately in developing country[5].

We collected 369 patients in China AIDS area and give them HAART for 1 year. We found: during the HAART, the declined trend of HIV RNA was almost similar in 3 groups (see graph 1), and there were obvious significant in HIV RNA between pre and post-HAART ($P<0.05$, see table 2). At the same time during the HAART, there were no differences in HIV RNA level among the 3 groups ($P>0.05$). Meanwhile, CD_4^+T lymphocyte counts elevated following HIV RNA declined, there were obvious difference between pre and post HAART(see table 1). Notably, we observed no statistically significant association even in rates and positions between the occurrence of the either HBsAg or HCV (see graph 3), that was, it was no effect on the immune reconstruction and anti-virus to AIDS. The above results were the similar as Cooper and Milles's reports[6]. But to the liver function, there were only 5.1% cases whose liver function was abnormal and rapidly recovered by themselves in a short time [9].

Otherwise, the knowledge about the interrelationship between these viruses and their effect on the immune system remains unclear[10]. Triple co-infected individuals are more likely to present with lower CD_4^+ counts and therefore reduced host immunity[4]. In our study, with the immune reconstruction and declination of HIV RNA, the reproduction of HCV and HBV was not effected (see table 3 and graph 2) and the level of those was almost in stable. This results were liked as others[7, 11], so there was no kinetic interactions in reproduction between HIV and HCV(or HBV)[12,13].

In other study, it has demonstrated that co-infection of HIV and hepatitis viruses (HBV and/or HCV) is on the increased and appears to decrease the CD_4^+ counts of patients who are coinfected especially with triple coinfection of HIV, HBV, and HCV[8]. Treatment of either hepatitis virus

is complex because of pharmacokinetic interactions with components of HAART regimens. In our results, the reproduction of HIV can be inhibited and the immune system can be reconstructed after HAART (AZT+DDI+NVP) to AIDS with HBV and (or) HCV co-infection. The HAART have no effect on liver function and even there were no resistant sites to 3TC and protease inhibitors (PIs) in China AIDS area (see graph 3). So the regimen of AZT+DDI+NVP is a suitable therapy for developing country and the other regimens containing 3TC or PIs, perhaps, also are good choices in China.

Acknowledgements: Authors are grateful to the Department of Pharmacology, National Key Technologies R & D Program of China during the 10th Five-Year Plan Period (No.2004BA719A13-05 and 2004BA719A10-123456) for financial support to carry out this work.

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10/27/2010

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Volume 8, Number 1, (Cumulative No.24) Part 3 February 15, 2011 ISSN:1097-8135

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