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## Historical Review on the Reproductive Technologies and Islamic Perspective

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**Abstract:** The new technologies in assisted reproduction has provoked considerable discussions and debates across all segments of human society. These revolutionary procedures in ART has probed the outermost boundaries of what is scientifically possible and acceptable. Micro manipulation at the very earliest stages of human development is a very delicate and sensitive issue with potentially explosive ethical, social, medico-legal and religious ramifications. Hence the turbulent and not uncommonly hostile controversies that has since evolved. The Islamic Organization for Medical Sciences (IOMS), first addressed this issue on human reproduction in May 1983. Human reproductive cloning, an offshoot of ART, which is currently attracting a lot of public and media attention was similarly addressed at this 1983 seminar. Since the IOMS seminar, there has been a multitude of medico-Islamic jurisprudence seminars to discuss various contemporary issues related to ART. This essay attempts to present historical review on the reproductive technologies and Islamic Perspective.

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**Keywords:** Historical Review, Reproductive Technologies, Islamic Perspective

### 1-Introduction

Dr. Edwards, an embryologist and Dr. Steptoe, a gynaecologist in the United Kingdom first pioneered the fertility technique called In Vitro Fertilisation Pre-Embryo Transfer (IVF – ET). In July 1978, they announced to the world the birth of the first test-tube baby, Louise Brown which was a landmark achievement in the science of reproductive medicine (1). Since then, a myriad of assisted reproductive techniques have surfaced, further refining and superseding earlier technologies. Assisted reproductive technologies (ART) refers to all the techniques involved in the management of infertility that require the handling and manipulation of gametes and embryos and treatment modalities to induce ovulation or spermatogenesis. Techniques of in vivo assisted reproduction facilitate the fertilization of the gametes within the reproductive tract of the wife. This may be achieved through the procedures of intrauterine insemination (IUI), intratubal insemination (ITI) and gamete intra fallopian transfer (GIFT) (2). Mating of the gametes occur extracorporeally during invitro assisted conception. The modalities to facilitate the fertilization invitro followed by transfer into the reproductive tract include in vitro fertilisation and embryo transfer (IVF - ET), pronuclear stage tubal transfer (PROST), zygote intrafallopian transfer (ZIFT) and tubal embryo stage transfer (TEST) (3,4,5). More radical forms of micro-manipulation techniques have been recently developed to assist fertilization of men with severe infertility. These include intracytoplasmic sperm injection (ICSI) and micro injection of round

spermatid nuclei into oocytes (ROSNI) (6,7) Since the introduction of IVF-ET technology, well over 500,000 babies have been born. The probability of a successful pregnancy is dependant on a variety of factors including the age and the reproductive health of the wife and the husband. Eventhough reported success rates from ART programs can be very confusing and misleading, the probability of a successful outcome has improved from virtually zero to 30-50% at ART centers worldwide. These new technologies in assisted reproduction has provoked considerable discussions and debates across all segments of human society. These revolutionary procedures in ART has probed the outermost boundaries of what is scientifically possible and acceptable. Micro manipulation at the very earliest stages of human development is a very delicate and sensitive issue with potentially explosive ethical, social, medico-legal and religious ramifications. Hence the turbulent and not uncommonly hostile controversies that has since evolved. The Islamic Organisation for Medical Sciences (IOMS), first addressed this issue on human reproduction in May 1983. Human reproductive cloning, an offshoot of ART, which is currently attracting a lot of public and media attention was similarly addressed at this 1983 seminar. Since the IOMS seminar, there has been a multitude of medico-Islamic jurisprudence seminars to discuss various contemporary issues related to ART. This essay attempts to examine the various bioethical facets of ART and present an Islamic perspective of the infertility problem and the bio-religio-ethics of ART.

### 1-1-Infertility In The Quran

There are a few case scenarios depicted in the Al-Quran which helps us to gain a proper insight into the problem of infertility. The first illustrates the story of Ibrahim SAW and his wife Sara as revealed in Surah 51: 28-30. "And they (angels) gave him (Ibrahim) glad tidings of a son endowed with knowledge. But his wife came forward clamouring, she smote her forehead and said; "A barren old woman!" They said, "Even so has thy Lord spoken and He is full of wisdom and knowledge." The aged Sara had willingly resigned to her destiny of being infertile but yet continued to be firm in her faith and true to her husband. She remained a complete, faithful woman in every other way. And she offered Hajar to Ibrahim SAW in marriage, so as to enable him to have children. She was ultimately blessed with a child, Ishaq SAW. As with the example of Ibrahim SAW, Zakaria SAW remained faithful and supportive of his infertile wife. In surah 21: 89-90, Allah says: "And (remember) Zakaria, when he cried to his Lord: "O my Lord! Leave me not without offspring, though Thou are the best of inheritors." So we listened to him and granted him Yahya. We cured his wife (barrenness) for him. These three were ever quick in emulation in good works; they used to call on Us with love and reverence, and humble themselves before us." Being infertile does not make one any lesser a man or woman. Like Zakaria SAW, one should beseech Allah for the blessings of offsprings. We pray and hope our children to perpetuate our family lineage and to continue to disseminate the teachings of God's chosen prophets. The example of the earlier prophets and their wives teaches one to pray to God and endeavour to rectify the infertility disorder but if one is unsuccessful then one accepts the predestination ordained as Allah has clearly decreed in surah 42: 49-40 "To Allah belongs the dominion of the heavens and the earth. He creates what He wills. He bestows (children) male or female according to His will. Or He bestows both males and females, and He leaves barren whom He wills; for he is full of knowledge and power." If the earlier two examples ended with the successful outcome of an offspring, the final scenario narrates the story of Asiya who never consummated her marriage to Pharaoh. She remained childless till her martyrdom but she nurtured a prophet, Musa SAW, right from his infancy. Asiya, though childless, remained full of faith and portrayed a shining example of faith and fortitude to all believing woman when struck with a similar predicament. Allah says in surah 66:11; "And Allah sets forth, as an example to those who believe the wife of Pharaoh: Behold she said: O my Lord! Build for me, in nearness to Thee, a mansion in the

Garden, and save me from Pharaoh and his doings, and save me from those that do wrong."

### 1-2-Infertility In The Hadith

Suffice it to quote two very instructive sayings of the Prophet SAW which would have a very significant impact on our handling of the infertility issue. "Marry women who will love you and give birth to many children for I shall take pride in the great numbers of my ummah" (Sunan al Nasai) In his deliverance of the message of Islam, the Prophet SAW produced a generation of Muslims who were torch bearers of the true teachings of Al-Quran, who has since been decorated with the accolade of the unique Quranic generation. And in this hadith he emphasized the importance of the large numbers of his ummah. Besides quality of the believers, quantity is similarly an important denominator. A pivotal principle taught by the prophet vis a vis medicine and reproductive medicine is no exception is contained in the following hadith reported by Sahih Muslim; "For every disease there is a cure" Infertility has been recognized by the World Health Organisation as a disease with significant mental and psychological morbidity. And quite clearly, the Muslims have been exhorted to explore the various curative strategies to overcome this disease which afflict up to 15% of couples.

### 1-3-Epidemiology Of Infertility In The Muslim World

The reported population of Muslims in 1992 was 1.25 billion (8). This statistic is expected to double in 2020. Up to 80 million of the world population are affected by some form of infertility problem. And Muslims contribute up to 40-50% of the infertile couples. The rate of infertility is relatively higher in the Muslim world when compared to the developed west. A WHO report have shown that the rate of tubal occlusion in Muslim sub Saharan Africa was well over three times that of other regions with the exception of the Eastern Mediterranean (9). The patterns of male infertility is however less apparent but regional variation in varicocele and other related condition have been reported.

### 2- Bio-Religio-Ethics Of Assisted Reproductive Technologies In The Muslim World

The teachings of the Quran and Hadiths have emphasized the vital role of the institution of marriage and the family structure. And inseparable from this is the act of procreation. To this effect Allah SWT says in surah 16: 72; "And Allah has given you wives of your own kind, and has given you, from your wives, sons and grandsons, and has made provisions of good things for you. Is it then in vanity that they believe and in the grace of Allah that they disbelieve?" It therefore follows that the prevention and treatment of infertility is encouraged

and becomes a medical priority because it will ensure an uninterrupted process of procreation (10). Islam enjoins the affected man and woman to seek medical treatment including contemporary ART, to fulfill their cherished hope of parenthood. Since marriage and purity of lineage is fundamental in the teachings of Islam, it is very important that this unadulterated inheritance of genes and heredity is preserved. Any method of ART practised must therefore guard against any mixing of the genes. Every newborn child must relate unequivocally to a biological and legal father and mother. Hence the premise to the practice of any modality of ART is to abide by the Shariah system of legally binding marriage through the union of the sperm and the ovum.

### **2-1-Assisted Reproductive Technologies-The Islamic Paradigm**

The Islamic Organisation for Medical Sciences (IOMS) first addressed this issue of assisted reproduction in their Fiqh Medical Seminar in May 1983. The IOMS seminar are always well attended by distinguished jurists, shariah experts, medical practitioners, scientists and specialists in other human sciences. Based on the conclusions of the Fiqh Medical Seminar of the IOMS and the opinions of other medical-shariah authorities in this area of ART, I have summarized the fundamental ground rules which must be adhered to diligently by any Muslim practitioner of ART. 1. The sanctity of the marital contract must not be violated at any point in time during the ART process. The Muslim practitioner of ART must guard jealously the purity and legality of the sperm and ovum of the couple. Since the union of the sperm and ovum is occurring one step beyond the act of sexual coitus, the fusion must take place within the jurisdiction of a marriage contract (11). 2. The dyad of the legal husband and wife must not be intruded by any third party. The involvement of a third person in the equation is totally unacceptable whether this take the form of a sperm, an ovum, an embryo or a uterus. Hence the widespread practice in ART facilities of sperm, ovum and embryo donation and the "rental" of uterus is incompatible with the Islamic injunctions related to human reproduction (11). 3. Once the marital contract has been terminated either due to divorce or death of the husband, assisted reproduction cannot be performed on the ex-wife or widow using sperm cells from the former or dead husband or using the previously cryopreserved embryos of the couple. The stored semen and cryopreserved embryo of her ex or dead husband now becomes alien to her and the latter to either party (12). 4. In ART programs, the excess pre-embryos produced can be frozen and stored in liquid nitrogen, a technique called cryopreservation. This can only be undertaken after the free informed consent of the

couple is obtained. The excess pre-embryos continues to remain the property of the couple. It can only be transferred to the uterus of the wife and only during the validity of the marriage contract (13). 5. Cryopreservation should only be allowed in specially designated sperm and pre-embryo banks or ART centres accredited by the relevant health authorities. An accurate and full proof system of documentation must be in place to guard against mixing of lineages and commercialism. Confidentiality of information should not be breached and tight security procedures should prevent unauthorized access to records (14). 6. Credentialling of all staff participating in the ART program is of utmost importance to ensure the highest standards of professionalism, trustworthiness, integrity and responsibility. Strict adherence to evidence based reproductive medicine, clinical practice guidelines and a code of ethical ART practice by all related professionals would help to protect the infertile couple who are often very desperate, emotional and hence very vulnerable to the unethical and profit driven ART practitioner. Hence the latter three qualities of trustworthiness, integrity and responsibility often supersede the string of professional qualifications (15) 7. Many countries have now restricted the numbers of eggs or embryos which could be placed in a woman in any one cycle. The recommended clinical guideline in Malaysia is that no more than 3 eggs or embryos can be implanted into a woman in any one cycle. However, up to a maximum of 4 eggs or embryos maybe transferred if two conditions are met; firstly, the patient has undergone no less than 2 ART attempts which were unsuccessful and secondly, the patient is between 35-45 years of age. If the procedures of stimulating and monitoring egg production are closely monitored and the numbers of embryos implanted are restricted, then the likelihood of generating excessive multifetal gestation would be minimized. However, if despite this, in excess of three foetuses are gestated, pregnancy reduction is permissible if the prospect of foetal viability is compromised or if the health or life of the mother is threatened. Multiple pregnancy of an order higher than twins presents an increased health hazard to the gravid mother and also for her fetuses which are more likely to be spontaneously aborted or to be delivered extremely prematurely with all the attendant complications of prematurity (16). 8. The abuse of infertility procedures maybe prevented and other related activities of ART maybe better monitored through acts of legislation. The Human Fertilisation and Embryology Act 1990 in the United Kingdom and Eire was an act of parliament which among others made provisions in connection with human embryos and any subsequent development of

such embryos and to prohibit certain practices in connection with embryos and gametes (17). The Ministry of Health is now at the stage of drafting laws to address the activities of ART in Malaysia. 9. There are various permutations of surrogate motherhood. In the first form, the surrogate is impregnated with the commissioning husband's semen; carry the pregnancy to term and give away the baby to the commissioning couple. The commissioning couple may via IVF transfer their resultant embryo to the surrogate mother. They therefore remain the biological parents. Or the surrogate may be impregnated with donated semen or embryo and reared by the commissioning parents. In this last case scenario, there will arise 3 set of parents; the rearing parents, biological parents and surrogate mother. A case was brought up in the legal courts recently about the parentage of a surrogate child. Five individuals laid claim to the child. And the court ruled that none had legal parental claims to the child! Surrogacy in all its forms is not allowed in Islam (18).

### 3-Pre-Implantation Genetic Diagnosis (PGD)

Two hadiths related from the Prophet SAW has helped us to have a better insight into the science of genetics. "Select your spouses carefully in the interest of your offspring because lineage is a crucial issue" "Do not marry your close relatives because you will beget weak offsprings" The second Caliph of Islam, Omar ibn El-Khattab, upon noting that a particular tribe intermarried with increased frequency, remarked to them: "You have weakened your descendants. You should marry strangers (people outside your tribe)".

The spirit of the exhortations of the Prophet SAW and his companion was to secure normal and healthy babies, protection of their early well being, endowed with the benefits of good genes from both parents and the prevention of congenital malformations and its consequent disabilities. A variety of inherited diseases may now be diagnosed in the pre-embryo stage prior to implantation into the uterus. Highly sensitive polymerase chain reaction (PCR) techniques have enabled the rapid amplification of minute amounts of DNA material from the embryonic cells. Fluorescent in situ hybridization (FISH) technology with combination chromosomal probes have made possible the genetic analysis of embryonal sex and various aneuploidies (19). Some of the potentially debilitating diseases which may be screened include Trisomy 13, 17 and 21, cystic fibrosis, haemophilia, Marfan's syndrome, incontinentia pigmentosa, x-linked immune deficiency, retinitis pigmentosa, fragile X syndrome, muscular dystrophy and Lesch-Nyhan disease. It is postulated that well over 200 diseases or conditions can be further isolated with ongoing PGD research

(20). The First International Conference on Bioethics in the Muslim World held in Cairo from 10-13 Dec 1991 examined very carefully this area of pre-embryo research (21). Collaborating this with the decisions of other scientific cum Islamic jurisprudence seminars, the following practice guidelines may be summarized:

Cryopreserved pre-embryos may be used for research purposes with the free and informed consent of the couple.

1. Research conducted on pre-embryos is limited only to therapeutic research. Genetic analysis of pre-embryos to detect specific genetic disorders is permissible. Hence diagnostic aids should be provided for couples at high risk for selected inherited diseases. The treated embryo may only be implanted into the uterus of the wife who is the owner of the ova and only during the span of the marriage contract.

2. Any pre-embryos found to be genetically defective maybe rejected from transfer into the uterus after proper counselling by the physician.

3. Research aimed at changing the inherited characteristics of pre-embryos (e.g. hair and eye colour, intelligence, height) including sex selection is forbidden.

4. sex selection is however permitted if a particular sex predisposes to a serious genetic condition. One of the first couple to use this technique of sex selection was hoping to escape a deadly disease known as x-linked hydrocephalus, which almost always affected boys. Embryonal sex selection would make possible the weeding out of other serious x-linked disorders including haemophilia, Duchenne muscular dystrophy and fragile X syndrome.

5. The free informed consent of the couple should be obtained prior to conducting any non-therapeutic research on the pre-embryos. These pre-embryos should not be implanted into the uterus of the wife or that of any other woman.

6. Research of a commercial nature or not related to the health of the mother or child is not allowed.

7. The research should be undertaken in accredited and reputable research facilities. The medical justification for the research proposal must be sound and scientific and conducted by a skilled and responsible researcher.

### 4-Conclusion(s)

Contemporary technology in the realm of assisted reproduction has been a major breakthrough in the management of infertility. Undoubtedly, it has brought great joy and happiness to many previously infertile couples. Along with it, ART has created her own set of bio-religio- ethical problems and

dilemmas. The response to this new technique in human reproduction has ranged from categorical condemnation by the Roman Catholic Church to the multiple reproductive permutations of the libertarian philosophy. It is interesting to note that in this very difficult, painful and emotional issue Islam has presented a middle of the road solution, moderating between the two extreme views. Allah says in surah 2: 143; "Thus we have appointed you a middle nation, that you may be witness against mankind, and that the messenger may be witness against you."

ART is Islamically acceptable and commendable provided it is practised within the husband and wife dyad during the span of the marital contract.

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## Sexual Behavior And Knowledge Of Aids & Other Stds: A Survey Of Senior High School Students

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**ABSTRACT:** The major objective of this study has been to examine the sexual behavior and level of knowledge of AIDS and other STDs of students at a senior high school. 116 students from the 9th to 12th grades provided information. Most of them believe that AIDS is the most serious disease the country faces. On the average, the students have modest knowledge on AIDS, but low level of knowledge on other STDs. The results show that over a third of the students have experienced sex, and most of them never use any protective means to avoid STDs.

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**Keywords:** Sexual Behavior, AIDS, STDs, High School Student

### 1-INTRODUCTION

Three or four years back, most people in Ethiopia never heard of AIDS. Those few who knew of AIDS felt that it was a problem of western societies where deviant sexual behaviors were practiced. It is now recognized, from a number of sero-surveys conducted since 1984, that HIV infection is also prevalent in Ethiopia. Sero-epidemiological surveys carried out in 23 towns since 1988 indicated that the prevalence rate of HIV infection was 16.9% among prostitutes, 13.0% among long distance truck drivers, 3.7% among blood donors, and 2.4% among scholarship winners (4). A sero-survey conducted in 1989 among prostitutes and long distance truck drivers in 8 towns (Adaitu, Asmara, Bahirdar, Diredawa, Metu, Nazareth, Nekemt, and Addis Ababa) has revealed that the prevalence rate in these towns increased by an average of over 60% in a year's time. Studies on Sill's also show that these are on the rise (1). The available evidence is clear in that the number of people infected with HIV will continue to grow until a vaccine and/or some other effective therapy for AIDS is discovered.

Until such time, however, the only practical means of halting the spread of HIV infection is through behavioral changes. Behavioral changes can come about with extensive Information, Education and Communication (IEC) programmes. As is the case with many other countries, such is the strategy adopted in Ethiopia to prevent the spread of the disease. The Ministry of Health (MOH) through its National AIDS Control Program (NACP) established four years ago, is entrusted with the responsibility of launching an effective IEC program (5).

Following the creation of NACP, extensive efforts have been made to inform and educate the public on HIV infection and AIDS. It has been some

time now since an educational campaign has been launched. Several conferences and/or workshops have been held to inform the public on the extent of the problem of AIDS. In view of the IEC campaign which is still underway, the aim of this study is to assess the level of knowledge of AIDS and other STDs (Sexually Transmitted Diseases) of young people at a senior high school level. The information for the study is drawn from a pilot study carried out in March, 1990.

### 2-METHODOLOGY

The objective of the study was to collective information on what students at the senior high school level know about AIDS and other STD's. It involved a total of 116 students from the 9th to the 12th grade, 30 each from grade 9, 10 and II and 26 from grade 12. Their ages ranged from 13 to 19 years, the majority (56.9%) being 15 and 16 (see table I).

The students from the 9th to the 11th grade were randomly selected from Kefitegna (Higher) 12 Senior High School (SHS). It was not possible to include 12th grade students from the same school, because at the time the study was carried out the students had already taken the ESLCE (Ethiopian School Leaving Certificate Exam), and were officially on vacation. Thus, volunteers from any other school were arbitrarily selected to participate in the study. Kefitegna 12 SHS became the study site because of easy access and cooperation of the school authorities.

Information was gathered from the respondents using a standard questionnaire prepared by SBG/GPA in Geneva translated into Amharic. The questionnaire was prepared to cover a wide area of interest including, among other things, background characteristics; sources of information on health disease etc.; knowledge of AIDS and STDs; and sexual behaviors.



Before the administration of the questionnaire the respondents were told about the importance of the study for the development of IEC programmes and thus the need to answer the questions honestly. They were assured of the confidentiality of their responses. The information gathered was processed through the Statistical Package for Social Science (SPSS).

There is a reasonable suspicion that there are variations among the background of students at the senior high school level in Addis Ababa. For instance, variations among students attending schools run by the state, religious institutions, and members of the international community. Such variations may have direct or indirect impact on how much knowledge and what attitudes students have on AIDS and STD's and, hence, on their sexual behaviors. As the bulk of the respondents for this study were selected from one government school (although most schools are operated by the state) the findings of this study may not be generalized to others.

Nevertheless, as there is a general lack of such studies that particularly focus on students at the senior high school level, the findings presented in this paper should provide a useful clue about what young people know, think and do in the face of a growing danger of AIDS. It is an attempt to fill a gap in information.

### 3-FINDINGS

#### 3-1-GENERAL INFORMATION

Roughly an equal number of boys (49.1 %) and girls (50.9%) have provided information. More or less equal numbers of boys and girls were selected from each grade level in order to have a reasonable mix of both sexes.

**Table 1.** Percent distribution of respondents by age and grade

Age	Grade				All Grades
	9th	10th	11th	12th	
13	33.3	-	-	-	3.4
14	16.7	3.3	-	-	5.2
15	50.0	46.7	23.3	-	31.3
16	13.3	33.3	43.3	11.5	25.9
17	3.3	3.3	23.5	15.4	11.2
18	3.3	6.7	3.7	42.3	13.8
19	-	6.7	3.3	30.8	9.5
Total	100	100	100	100	100
N	(30)	(30)	(30)	(26)	(116)

89.6% of the respondents were Christians, 5.2% Moslem and 5.2% others. 63% of, them either strongly agree and/or agree that AIDS is the most serious disease facing the; country, while 28.4% are not sure and 7.7% do not agree.

37.9% (44) reported that they were sexually active currently. Of the sexually active respondents, 30 were boys comprising nearly 53% of the total male respondents. 14 girls admitted to be sexually active and these constitute about 24% of the female respondents.

Comparing the proportion of sexually active boys with girls, one might raise a question as to why fewer girls than boys reported being sexually active. This might suggest two things: Ethiop.J.Health Dev. Vol.4 No.2,1990 I) that girls perhaps do not normally admit sexual practices for cultural reasons, and 2) that boys at these ages and grades are perhaps more prone and daring to engage in sexual practices than girls.

**Table 2.** Distribution of respondents who are sexually active by number of sex partner

No. of sex partners	Sex		Both sexes
	Boy	Girl	
1 only	-	7.1	2.3
2 to 3	27.6	35.7	30.0
4 to 5	27.5	35.7	30.2
6 to 7	10.3	14.3	11.6
8 and above	34.4	7.1	25.6
Total	100*	100*	100
N	(29)**	(14)	(43)

\* Does not add "" to 100 due to rounding off

\*\* 1 boy did not respond

The ages of the respondents at the time of first sex ranged between 12 and 18 years. Most (70.5%), however, had first sex between the ages of 14 and 16. The number of persons they have had sex with ranges between 1 and 5. Except one girl, all who are sexually active have had sex with more than one person. 60.2% of the respondents (over 70% of the girls and 55% of the boys) have reported to have had sexual relations with 2 to 5 persons. Over a third of the boys had sex with eight and more persons.

The respondents who are sexually active were asked how often they and/or their sexual partners used a protective method, such as condoms, to avoid catching AIDS, getting other Sills, and being pregnant. The responses are given in table 3.

It is interesting to note from table 3 that the majority of the respondents (boys as well as girls) used no protection at all. Most of the girls use protection to avoid pregnancy only sometimes. The table also depicts that down the list of priority is protection against AIDS. About 82% of the respondents (76.7% and 92.9% of the boys and girls respectively) never used protection against AIDS.

#### 3-2-SOURCES OF INFORMATION ON AIDS AND OTHER

The respondents were asked whether radio and/or television sets were available at home. All, except one, have a radio set, while only 40.5% have TV. They were also asked what their major sources of information about AIDS and STDs were. They were given a long list of sources of information among which they were asked to choose the first and second major sources of information. Table 4 depicts the responses obtained.

On the 1st count radio (33.6%), medical institution/personnel (18.9%), and TV (16.4%) followed by newspapers (22.4%), medical institutions/personnel (20.7%), and TV (19.8%) were the three most important sources of information about AIDS.

Major sources of information about other STDs included radio (14.7%), medical institutions/personnel (14.1%), and teachers/schools (10.3%), followed by medical institutions/personnel (28.5%), teachers/schools (9.5%), and radio, newspaper, and TV (7.85% each).

The mass media (which constitutes the first five items in the table) has been instrumental in the

dissemination of information on AIDS and other STDs. 63.8% and 58.6% of the respondents mentioned the mass media as their 1st and 2nd major sources of information about AIDS respectively. With regard to other STDs, 7.1% and 30.3% stated the mass media as the 1st and 2nd major information source respectively.

An interesting observation from Table 4 is the relatively marginal role the family (including items 8 to 11), religious institutions, and educational institutions play in the dissemination of information about AIDS and other STDs. One would have thought that these social institutions, being the main agents of socialization, will have paramount contribution to the development of the knowledge of young people regarding major concerns such as AIDS.

The findings indicate the contrary. This is perhaps a reflection of the conservative nature of these institutions in Ethiopia in matters of discussing sex with young people on the one hand and their own lack of general knowledge about AIDS and other STDs.

**Table 3.** Percent distribution of use of protection to avoid catching AIDS, other STD and pregnancy by sex

Response	AIDS			Other STD			Pregnancy		
	1	2	3	1	2	3	1	2	3
Never	76.7	92.9	81.8	53.3	57.1	54.5	56.0	28.0	47.7
Sometimes	16.7	-	11.4	40.0	42.1	40.9	30.0	71.4	43.2
Mostly	3.3	7.1	4.5	3.3	-	2.3	13.3	-	9.1
Always	3.3	-	2.3	3.3	-	2.3	-	-	-
Total	100	100	100	100	100	100	100	100	100
N	(30)	(140)	(440)	(30)	(14)	(44)	(30)	(14)	(44)

1 = Boys 2 = Girls 3 = Both sexes

### 3-3-KNOWLEDGE OF AIDS

A total of 22 questions were asked to measure the respondents' knowledge about AIDS. These questions included what the mode of transmission of the disease are, its symptoms, its effects, behaviors that increase the risk of the disease and preventive methods. The full content of the questions is listed in Annex, along with the frequency distribution of the responses to each question. The respondents were given '1' point if they answered a question correctly and '0' if not. A composite score, including the 22 questions, was developed to reflect the overall knowledge of the respondents.

The findings indicate that 32.6% of the respondents could not answer half of the questions correctly, while none of them gave correct answers for over 65% of the questions.

Nevertheless, the majority of them (67.3%) have answered more than half of the questions. The overall average knowledge score is 10.85, i.e., a correct

response of slightly less than 50% of the questions. On the whole the result implies that the respondents have a somewhat modest knowledge of AIDS. The mean knowledge of AIDS score by sex and grade is given in table 5.

Table 5 classifies the respondents according to two variables, sex and grade. Such a classification allows us to compare the two variables with respect to the level of knowledge of AIDS. Furthermore, mean scores on knowledge within a given grade can be compared across sexes, and within a given sex can be compared across grades.

Looking at the means in Table 5, one might wonder whether the observed differences can be attributed to chance or whether sex and grade have any effect on the score. One of the statistical procedures commonly used to test whether there is significant difference in means is through the ANOVA (Analysis of Variance) (2). ANOVA was performed taking Knowledge of AIDS score as the

dependent variable and sex and grade as the independent variables. The result indicates that the means (for boys and girls as well as for the different grade levels) do not differ in a significant way ( $F_{7,108}=0.977$ ). Other independent variables, such as religion and sexual experiences (whether the respondent is sexually active or not) assumed to affect the dependent variable were added along with sex and grade in the ANOVA, and the result indicates that the means are not significantly different ( $F_{7,105}= 1.050$ ). The finding, therefore, suggests that the respondents have more or less the same level of knowledge irrespective of sex, grade, religion and sex experience.

A further examination of the frequency distribution (see Annex 1) of the responses for each of the knowledge of AIDS questions reveals some of the misconceptions and strong sides of the

respondents. A substantial proportion of the respondents have a common misconception that HIV is transmitted by a mosquito carrying infected blood (79.3%) and by wearing clothes used by someone who has the AIDS virus (37.1 %). 49.1 % have the misunderstanding that if a test for AIDS is negative it means that one cannot get AIDS in the future, while a good proportion of them believe that AIDS can be cured if diagnosed early (44%) and that a vaccine is discovered which can prevent people from getting AIDS (44%). A large majority of them, nevertheless, quite correctly know that a person can catch AIDS by having sex with someone who has the AIDS virus (93.1 %), that the chances of becoming infected with the AIDS virus are reduced by having sex with only one faithful partner (86.2%) and that the risk of being infected with HIV is increased by having many sex partners (87.9%).

**Table 4.** Percent distribution of respondents by 1st and 2nd major sources of information about AIDS other STD

Source of information	AIDS		Other STD	
	1st	2nd	1st	2nd
1. Radio	33.6	9.5	14.7	7.8
2. Newspaper/magazines	6.9	22.4	6.9	7.8
3. Pamphlet/handouts	1.7	2.6	1.7	.9
4. Books/journals	5.2	4.3	8.6	6.0
5. TV	16.4	19.8	5.2	7.8
6. Movies	2.6	.9	.9	.9
7. Videos	-	.9	-	.9
8. Mother	-	-	5.2	.9
9. Father	-	1.7	-	3.4
10. Brothers/Sisters	-	-	1.7	-
11. Other family members	1.7	-	3.4	.9
12. Friends/classmates	1.7	.9	2.6	-
13. teachers/School personnel	2.6	3.4	10.3	9.5
14. Health institutions/health personnel	18.9	20.7	14.1	28.5
15. church/mosque/religious leaders	.9	.9	-	.9
16. Others	-	1.7	5.2	3.4
17. None	5.2	1.9	3.4	3.4
18. Missing	2.6	9.5	6.0	17.2
Total	100	100	100	100
N	(116)	(116)	(116)	(116)

**Table 5.** Mean knowledge score by sex and grade

Sex	Grade				Means
	9th	10th	11th	12th	
Male	10.71 (14)*	11.60 (15)	11.13 (15)	10.15 (13)	10.93 (57)
Female	11.50 (16)	10.53 (15)	11.07 (15)	9.85 (13)	10.78 (59)
Means	11.13 (30)	11.07 (30)	11.10 (30)	10.00 (26)	10.85 (116)

\*Figures in parenthesis are number of respondents in each category.

**Table 6** mean STD score by sex and grade

Sex	Grade				Means
	9th	10th	11th	12th	
Male	3.20 (14)*	3.10 (15)	3.04 (15)	3.42 (13)	3.18 (57)
Female	3.26 (16)	3.06 (15)	3.33 (15)	3.02 (13)	3.17 (59)
Means	3.23 (30)	3.08 (30)	3.19 (30)	3.22 (26)	3.18 (116)

\* Figures in parenthesis are number of respondents in each category

### 3-4-KNOWLEDGE OF OTHER STD's

The respondents were asked if they had heard of sexually transmitted diseases (STD) such as gonorrhea, syphilis, and genital herpes. All of them had heard of the first two, while none had heard of the third. In order to measure knowledge of STDs, the respondents were given 7 statements to which they responded. These included how STDs are transmitted, their effects, and methods of prevention. The full list of the statements is given in Annex 2 along with the frequency distributions of the responses to each of the statements. They were given a Likert scale of five fixed alternative responses, ranging from 'definitely true' to 'definitely false', to choose from. If respondents correctly responded 'definitely true' or 'definitely false' to a statement they were given 5 points, 4 points if they correctly responded 'probably true' or 'probably false', 3 points for uncertain, undecided or do not know responses, and 2 points and 1 point respectively if they responded 'definitely true' and 'definitely false' incorrectly. The points for each of the 7 statements were then added up and divided by 7 to establish composite STDs score to reflect the respondents over all knowledge of STDs. A respondent who gave correct answers for the 7 statements scored 5 points, while the vice versa obtained 1 point.

The findings indicate that the majority of the respondents (69% ) did not know and/or were uncertain of STDs. Only 20.7% have somewhat adequate knowledge of STD while 10.3% have wrong perception. The overall average STD score is 3.18, a middle position expressing uncertain and/or do not know responses. The distribution of the mean knowledge of STD score is given in Table 6.

The means displayed in Table 6 seem to be similar , both across sex and grade. A mean score of 3 points, expressing uncertain or do not know responses, is more or less evenly distributed in each of the categories. The result implies that on the average the respondents do not have a clear understanding of STDs.

The ANOV A result also shows that there is no statistically significant difference in the means when STD score is taken as the dependent variable and sex

and grade are taken as the independent variables. This means that sex and grade do not have statistically significant effect on the knowledge of STD score ( $F_{7,108} = 1.456$ ). The result was statistically insignificant when additional explanatory variables' religion and sex experience, were included along with sex and grade as the independent variables ( $F_{7,105} = 0.673$ ). The finding, thus, implies that the respondents across the board, i.e., irrespective of whether they are girls or boys, lower or higher grades, Christians or Moslems, did not have adequate knowledge of STDs.

A close observation of the responses furnished by the respondents (see Annex 2) indicates that most of them admitted to having no knowledge with regard to each of the STD questions. 52.6% either do not know and/or do not believe that the use of protective methods such as condoms can help avoid getting STDs. On the other hand, 69% either do not know and/or erroneously believe that people who had STD earlier cannot catch it again. A large majority either do not know (35.3%) and/or have a misconception (42.3%) that gonorrhea can be transmitted from toilet seats. 71.6% did not know whether or not there is a cure for genital herpes.

### 4-DISCUSSION

The results of this study have generated useful information concerning what young people at one senior high school know, think and do at a time when the danger of AIDS is becoming an important agenda of discussion. The major source of information on AIDS, (63.8%) and other STDs (58.8%) for the respondents is the mass media, of which radio is the most important. Most (68%) believe that AIDS is the most serious disease facing the country at present. The finding has revealed that the students on the average have a modest (score of 10.85 out of a maximum of 22 points) knowledge of AIDS. Knowledge of STDs is low (score of 3.18 on the average) in that most (69%) have taken a 'do not know' position.

Across the board, the respondents 1 knowledge of AIDS and other AIDS is more or less similar. The respondents' sex, grade, religion, and current sex experience do not seem to have significant effect on

their knowledge of; AIDS and other STDs. This is unexpected particularly in view of the assumption that one would expect more knowledge at least with an increase in grade levels, which is also correlated with age. The findings in this study do not confirm this expectation. Although the majority (62.1%) have had no sexual relationship yet, a substantial proportion (37.9 %) are sexually active. Almost all of the sexually active have had sex experience with more than one sex partner, the majority (60.2%) having experience with 2 to 5 partners. Moreover, most of them either never and/or only sometimes use protective methods against AIDS, other STDs and pregnancy. The figure is more so against AIDS (76.7% for boys and 92.9% for girls), which is down the list of their priority. It is quite puzzling to learn that almost all of the girls who are sexually active either never and/or only sometimes used protection against pregnancy.

Such type of behavior is detrimental to the young people over time and puts them at risk. The evidence suggests that with such cautionless sexual behaviors (with respect to the already sexually active) and not too adequate knowledge of AIDS and STD, the students most likely to be vulnerable to HIV infections.

In view of this, much more than what is being done is expected of IEC programmes in the way of developing young people's knowledge of AIDS and other STDs, and hence changing their sexual values. The strategy of changing young people's sexual behaviors with a view to creating sexual values that reduce the risk of AIDS and other STDs should not be limited to conveying relevant information through the mass media. The dissemination of information to young people should be expanded by incorporating other important institutions, such as the family,

educational and religious institutions. These institutions, which function as basic socializing agents, need to assume the responsibility of creating desirable sexual values in young people. The evidence in this study has revealed that the role of these institutions has been insignificant so far.

Among other things, the role of the educational institutions in the dissemination of IEC should be strengthened possibly through the introduction of effective sex education at least at the high school level. Moreover, IEC programmes should aim at encouraging parents and/or other older family members to discuss and influence their children and/or younger brothers and sisters sexual behaviors. This should, perhaps, be underscored particularly in view of the conservative nature of most Ethiopian families in matters of sex. The role of religious institutions, where moral values are instilled in the general population, should not be underestimated. The contribution of these institutions, however, will depend on how IEC programmes reach and equip them with the knowledge required to carry out the task.

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ANNEX 1. Frequency distribution of respondents for 22 questions of knowledge of AIDS by correct and incorrect responses

Questions	Correct %	Incorrect %
1. Most people with AIDS virus will develop AIDS	76.7	21.5*
2. Can get AIDS by shaking victims hands	83.6	16.4
3. A person can be infected with the AIDS virus and not show signs of disease for many years	69.8	30.2
4. Most people with AIDS will die of the disease.	82.6	12.9
5. Can catch AIDS by sharing needles/syringes with someone who has the AIDS virus.	83.6	16.3
6. A person who looks healthy but has the AIDS virus can pass it onto other people.	73.3	26.8
7. A person can get AIDS by having sex with someone who has the AIDS virus.	93.1	6.9
8. A pregnant woman who has the AIDS virus can pass it onto her baby.	86.2	13.8
9. There is no cure for AIDS	64.7	34.5
10. A person can get AIDS by wearing clothes used by someone who has the AIDS virus.	62.9	37.1
11. AIDS can be cured if diagnosed early.	56.0	44.0
12. The chances of being infected by the AIDS virus are reduced by having sex with only one faithful partner.	86.2	12.9

13. A person can get AIDS by donating blood.	62.9	36.2
14. Doctors have discovered a vaccine which can prevent people from getting AIDS.	56.0	44.0
15. Having many sexual partners increase a persons risk of being infected with the AIDS virus	87.9	12.0
16. There are tests that can show if a person has the AIDS virus.	83.6	16.4
17. A person can get AIDS by being bitten by a mosquito which has already fed on a person with AIDS.	20.7	79.3
18. You can tell if a person has the AIDS virus by his or her looks.	40.5	58.6
19. Use of a condom when having sex can prevent AIDS.	60.3	40.9
20. The chances of becoming infected with the AIDS virus are increased by having sex with someone you do not know very well.	55.2	43.1
21. Once infected with the AIDS virus a person can infect others for the rest of his/her life	78.4	20.3
22. If you take an AIDS test and it is negative it means that you can not get AIDS in the future.	49.1	49.1

\*Percent does not add up to 100 due to missing cases.

#### ANNEX 2. Percent distribution of respondents for 7 statements of knowledge of STD

Statements	Responses				
	5	4	3	2	1
1. You can get syphilis from some one who does not show symptoms of the disease.	27.6	25.9	25.9	7.8	7.8*
2. Gonorrhea can be prevented by using condom.	12.9	24.1	28.4	10.3	18.1
3. You can get gonorrhea from toilet seats.	20.7	21.6	35.3	6.0	14.7
4. There is cure for genital herpes	3.4	6.9	17.6	9.5	2.6
5. Untreated syphilis can lead to sterility.	37.9	19.0	26.7	8.6	7.8
6. If a person had STD (like syphilis) he/she cannot catch it again.	18.1	19.0	31.9	10.3	19.8
7. One can avoid getting STD like syphilis by using a condom.	24.1	18.1	29.3	5.2	18.1

\*Percent does not add up to 100 due to missing cases.

#### KEY

s = Definitely True

4 = Probably True

3 = Uncertain/Do not know

2 = Probably False

1 = Definitely False

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## Serum Autoantibodies in Chronic Hepatitis C: Comparison with Hepatitis C/Autoimmune Hepatitis Overlap Syndrome in Egypt

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**Abstract: Background:** Hepatitis C virus—autoimmune hepatitis (HCV/AIH) overlap syndrome had been described in the literature since the early 1990s with numerous case reports and proposed guidelines of management. However, definitive diagnosis for the syndrome remains controversial and there are still no formalized treatment strategies. The aim of this study was to test how hepatitis C virus (HCV)-associated autoantibodies differs from those of AIH in terms of titer and sub specificities and to find the best combination of antibodies in discrimination between the two conditions. **Methods:** Liver biopsy and blood samples were taken from clinically and serologically confirmed patients with chronic HCV infection (n=57) and patients suspected to have HCV/AIH overlap syndrome (n=21). HCV infection was determined by detection of anti-HCV antibodies using a third-generation enzyme immunoassay and active virus replication defined by quantitative measurement of HCV RNA. Antinuclear antibodies (ANA) and its pattern, anti-smooth muscle antibodies (ASMA), Anti-Actin antibody (AA), Antimitochondrial antibody (AMA), anti liver-Kidney microsome-1(anti-LKM1) and perinuclear staining of antineutrophil cytoplasmic antibodies (p-ANCA) were detected by immunofluorescence assay. Anti-soluble liver antigen (SLA) was measured by ELIZA. Serum protein electrophoresis was done for gamma globulin measurement. **Results:** Statically significant difference regarding serum autoantibodies positivity and subspecificities were evident between chronic hepatitis C and HCV/AIH overlap syndrome patients. Elevated  $\gamma$ -globulins was the best test for selecting HCV/AIH overlap syndrome patients out of chronic HCV patients, followed by anti-actin, atypical p-ANCA, the homogeneous ANA pattern, the worse was ASMA. Positivity for AMA, anti-LKM-1 and SLA antibodies were not observed in all patients sera. The best combination was homogeneous ANA, anti-actin SMA and p-ANCA with sensitivity 85.71%, specificity 73.68%, and accuracy 76.92%. **Conclusions:** Serum autoantibodies positivity and sub-specificities should be used in differentiation between chronic hepatitis C and HCV/AIH overlap syndrome. The best combination was homogeneous ANA, anti-actin SMA and p-ANCA.

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**Keywords:** HCV, AIH, overlap syndrome and autoantibodies.

### 1. Introduction

Worldwide, 130–170 million persons are living with chronic hepatitis C virus infection [1] which, if left untreated, can result in cirrhosis and liver cancer. Egypt has the largest burden of HCV infection in the world, and incidence rates have been estimated at 2.4/1000 person-years (165,000 new infections annually) [2]. Hepatitis C is the main cause of liver-related morbidity and mortality and represents a worldwide public health problem [3]. Chronically infected HCV frequently leads to autoimmune response including the production of autoantibodies and the coincidence of autoimmune diseases [4]. The diversity of autoantibodies in particularly non-organ-specific autoantibodies has been widely established in sera of patients with HCV-related chronic liver disease. Anti smooth muscle (SMA) and anti- nuclear (ANA) antibodies have been

detected in approximately one third of the cases [5,6], while antibodies to liver/- kidney microsomes type 1 (anti-LKM1) have been found rarely (from 0% to 5%) [6, 7]. In addition to these “conventional” autoantibodies, patients with AIH have a wide range of other autoantibodies. Those most frequently found include perinuclear staining antineutrophil cytoplasmic antibodies (p-ANCA), and soluble liver antigen (SLA) [8].

Hepatitis C virus—autoimmune hepatitis (HCV/AIH) overlap syndrome has been described since the early 1990s [9]. Following the identification of HCV/AIH overlap syndrome, reports began to appear that a high proportion of patients who otherwise fulfilled criteria for AIH had evidence of HCV infection, suggesting that this virus might be an aetiological factor in the development of AIH[10].

The diagnosis of AIH is based on the revised descriptive criteria for diagnosis of AIH reported by the International Autoimmune Hepatitis Group (IAHG) in 1999[11]. Whether the application of the AIH scoring system is useful in cases of AIH/overlap syndromes and furthermore, in cases of AIH with concurrent other liver disease has not been extensively validated so far[12]. However, some of the features that may support AIH diagnosis, such as elevated serum IgG, detection of autoantibodies and histologically evident interface hepatitis, can occur with variable frequency in a wide range of other liver disorders [13]. The problem arising in diagnosis makes the decision for clinical management difficult as corticosteroids, which constitute the treatment of choice in AIH are usually contraindicated in HCV as they can induce exacerbation of viral hepatitis, and the presence of AIH appears to predispose to severe adverse reactions during antiviral treatment with interferon-alpha. Such patients need to be treated with caution for the underlying diseases [12].

The aim of this study was to test how hepatitis C virus (HCV)-associated autoantibodies differs from those of AIH in terms of titer and sub specificities and to find the best combination of antibodies in discrimination between the two conditions.

## 2. Materials and Methods

This was a cross-sectional case control study that included 78 patients attending an HCV out-patient clinic at Ain Shams University Hospital. Studied patients included 57 with chronic HCV infection and mean age  $\pm$  SD of 31.6 $\pm$ 0.8 years and 21 suspected to have HCV/AIH overlap syndrome with a mean age  $\pm$  SD of 32.2 $\pm$ 0.9 years. Informed consent was obtained from all subjects with approval granted by Ain shams Research and Ethics Committee "Ain-shams faculty of medicine federal number IRB00006444, prior to sample collection. The diagnosis of chronic hepatitis C (HCV) was based on clinical and laboratory evaluation. Serologic evidence of chronic HCV infection as determined by detection of antibodies to HCV (anti-HCV) using a third-generation enzyme immunoassay (Abbott Diagnostics) and active virus replication as defined by the detection of HCV RNA using a quantitative HCV RNA serum detection using (Amplicor; Roche Diagnostic Systems). None of the patients had ever received treatment with pegylated interferon and/or ribavirin.

Complete data for calculation of the revised IAHG score were reviewed retrospectively [11]. A pretreatment score of 10 points or higher, indicate "probable" AIH. A pretreatment score of 15 points, indicate "definite" AIH. Exclusion criteria included patients with hepatitis B infection, excluded on the basis of immunoassay negativity for serological markers (HBsAg, HBeAg, anti HBs, anti HBc, anti

HBe, and PCR DNA for HBV). Intake of alcohol or potentially hepatotoxic drugs was ruled out in all patients.

All patients were submitted to the following: full history taking, thorough clinical examinations with record of any sign of cirrhosis or autoimmunity, full blood chemistry including complete liver function tests, liver biopsy and abdominal ultrasonography to detect ascites and/or liver cirrhosis.

### Immunofluorescence measurements:

The standard indirect immunofluorescence (IF) technique was used for the detection of AMA, ASMA, anti-actin and anti-LKM-1 using a composite substrate comprising liver, kidney and stomach from rodents (DiaSorin Inc, USA) with titer  $\geq$  40 considered positive results[14]. ANA test was performed by IF technique on HEp-2 cells (DiaSorin Inc, USA) with a starting dilution of 1/40 [15]. Atypical p-ANCA was determined by the method of Terjung *et al.*, [16] using fixed neutrophils (Immco, USA) at a dilution of 1/20.

### Anti- soluble liver antigen (SLA) measurement:

Anti- SLA was detected by enzyme linked immunosorbent assay (ELISA) kit (Inova diagnostics Inc, USA) We had investigated reactivity of SLA positive sera against  $\alpha$ -enolase and tRNP(Ser) sec using rat and primate liver homogenate and the recombinant antigens).

### Biochemical measurements:

Alkaline phosphatase, ALT, AST and total protein were measured by commercially available kits (Randox-UK).

### Gamma globulin measurement:

Serum protein electrophoresis was performed according to the method of Alper [17]. Level of protein fractions can be estimated by measuring the total serum protein and then multiplying that by the relative percentage of each protein fraction.

### Statistical analysis;

Data was analyzed using SPSS statistical package version 15. Numerical data were expressed as mean, standard deviation. Qualitative data were expressed as frequency and percentage. Correlation between different antibodies frequencies were evaluated by Pearson's  $\chi^2$  test. Comparison of continuous variables was made by means of the Student's t test.

## 3. Results:

Comparing patients with probable AIH along HCV (HCV/AIH) overlap syndrome with the patients with only chronic viral infection, It was found that patients with AIH / HCV had significant higher levels of AST and ALT, and lower levels of ALP and low HCV load than the patients with only viral pattern ( $p < 0.001$ ). On the other hand, higher serum gamma globulin was observed among patients with HCV/AIH patients in



comparison to those with HCV infection ( $p < 0.001$ ). Non-significant difference was found regarding age and gender (Table 1).

Liver biopsy of chronic HCV patients showed, mild portal inflammation with lymphoid aggregates, mild periportal piecemeal necrosis, steatosis, bile duct damage, and apoptosis. Portal fibrosis was detected in all of our patients in both groups. Patients with HCV/AIH overlap syndrome had mixed histological findings of chronic HCV with one of the following: Interface hepatitis (5 patients 23.8%), lymphoplasmocytic infiltrate in the portal tracts (10 patients 47.6%), or hepatic rosette formation (6 patients 28.6%) (Table 2).

Compared with HCV/AIH overlap syndrome, HCV-associated ANA and SMA exhibited ANA-

homogenous pattern and SMA-anti actin antibodies at a lower prevalence (0% with median titer 80(40-160) Vs. 47.61% with median titer 320 (160-640) and  $P < 0.001$ ) and (0% with median titer 40(40-80) Vs. 57.14%, with median titer 320(320-640) and  $P < 0.001$ ) respectively. While p- ANCA positivity was detected in 24.56% of HCV patients and 52.38% of HCV/AIH overlap syndrome ( $P = 0.004$ ). Positivity for AMA, anti-LKM-1 and SLA Abs were not observed in all patients sera (Table-3).

Using autoantibodies in different combinations revealed that the best results were obtained when combining Homogeneous ANA, anti-actin SMA and p -ANCA with sensitivity 85.71%, specificity 73.68%, and Accuracy 76.92% (Table 4).

**Table 1: Comparison between patients with chronic HCV and patients with HCV/AIH overlap syndrome**

Parameter	Chronic HCV (n=57)	HCV/AIH overlap syndrome (n=21)	p-value
Age	31.6±0.8	32.2±0.9	0.58
Sex (M/F)	39/18	15/6	0.983
AST (U/L)	158.29± 10.22	173.61± 6.85	<0.001
ALT (U/L)	142.29± 4.65	164.1± 6.69	<0.001
ALP (U/L)	89.8± 0.50	73.2±0.44	<0.001
PCR (IU/mL)	846587.75±3878.25	451786.46± 2877.71	<0.001
$\gamma$ globulin $\geq 1.5$ (g/dl)	0/57	21/21	<0.001

M/F: Male/female, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline phosphatase, ANA: Antinuclear antibodies

**Table 2: Liver biopsy in patients with chronic HCV and patients with HCV/AIH overlap syndrome**

Parameter	Chronic HCV (n=57)	HCV/AIH overlap syndrome (n=21)
Fibrosis	57(100%)	21(100%)
Portal-periportal necroinflammation	55(96.4%)	20(95.23%)
Lobular necroinflammation	50(87.71%)	18(85.71%)
Interface hepatitis	0(0%)	5(23.8%)
Intense plasma cell infiltrate	0(0%)	10(47.61%)
Rosettes	0(0%)	6(28.57%)

**Table (3): Serum ANA, ASMA and p ANCA in Chronic HCV: A Comparison with HCV/AIH overlap syndrome**

Parameter	Chronic HCV (n=57)	HCV/AIH overlap syndrome (n=21)	p-value
ANA titer* Homogeneous ANA	ANA-Positive chronic HCV (44/57 patients)	ANA-Positive HCV/AIH overlap (20/21 patients)	<0.001
	80 (40-160)	320(160-640)	<0.001
	0 (0%)	10(47.61%)	
ASMA titer* Anti-actin ASMA	ASMA-Positive chronic HCV (34/57 patients)	ASMA-Positive HCV/AIH overlap (16/21 patients)	<0.001
	40 (40-80)	320(160-640)	<0.001
	0 (0%)	12(57.14%)	<0.001
p-ANCA	14(24.56%)	11(52.38%)	<0.001

\*Reciprocal of the dilution; median (range).

ANA: Antinuclear antibodies, ASMA: Anti-smooth muscle antibodies and p-ANCA: perinuclear anti-neutrophil cytoplasmic antibody

**Table (4): Value of combined use of autoantibodies in discrimination between Chronic HCV and HCV/AIH overlap syndrome**

Parameter	Sensitivity	specificity	PPV	NPV	Accuracy
ANA	95.24%	22.81%	37.04%	92.86%	42.31%
Homogenous pattern	47.62%	100%	100%	83.82%	85.89%
ASMA	76.19%	40.35%	32%	82.14%	50%
AA-ASMA	57.71%	100%	100%	86.36%	88.46%
p-ANCA	52.38%	75.44%	44%	81.13%	69.23%
ANA + ASMA	90.48%	38.80%	35.19%	91.67%	52.56%
Homogenous pattern +ASMA	90.48%	38.60%	35.19%	91.67%	52.56%
Homogenous pattern +AA-ASMA	76.19%	100%	100%	91.94%	93.59%
Homogenous pattern +p-ANCA	76.19%	73.68%	51.61%	89.36%	74.36%
AA-ASMA+p-ANCA	71.43%	75.44%	51.72%	87.76%	74.36%
Homogenous pattern +AA-ASMA +p-ANCA	85.71%	73.68%	54.55%	93.33%	76.92%

ANA: Antinuclear antibodies, ASMA: Anti-smooth muscle antibodies, AA: Anti-actin and p-ANCA: perinuclear anti-neutrophil cytoplasmic antibody. PPV: Positive predictive value. NPV: Negative predictive value.

#### 4. Discussion:

The diagnosis of true HCV-AIH overlap syndrome is often challenging, as the concurrent presence of serologic markers typically found in AIH and serologic evidence of HCV infection is well documented [5,18]. Autoantibodies such as antinuclear antibody, anti-smooth muscle antibody, or anti—liver and kidney microsomal 1 antibody have been reported in 9-38%, 5-91%, and 0-10%, respectively, of patients with chronic HCV infection [19]. Therefore, in order to definitively diagnose HCV-AIH overlap syndrome as a distinct entity, the existence of both diseases must be confirmed independently.

In the current study, on comparing autoantibody profile of HCV/AIH overlap syndrome patients with those in chronic HCV infection patients. Associated ANA, ASMA, and AA-ASMA exhibited a higher prevalence (95.24% vs. 59.65%, 76.19% vs. 59.65%, and 57.71% vs. 0%) respectively while, the concomitant positivity of ANA-Homogenous pattern and AA-ASMA was evident in 76.19% of HCV/AIH overlap syndrome patients and never in the sera of chronic HCV patients. The prevalence of autoantibodies detected in the present study was substantially in accordance with previous reports [20,21] which used the same immunofluorescence antibody screening dilution. Also, Rigopoulou and Dalekos [22] reported that the homogeneous ANA pattern is one of the Features of AIH in patients with chronic HCV.

The American Association for the Study of Liver Diseases has recommended the use of atypical p-ANCA for diagnosis of AIH since, occasionally, it may be the only autoantibodies present [23]. Indeed, it must be noted that p-ANCA in patients with AIH (atypical pANCA) differ from the classical p-ANCA by retention of the perinuclear staining (produced

primarily on ethanol fixed cells) on formaldehyde fixed cells. Zauli *et al.* [24] found atypical p-ANCA in 65% of patients with AIH and in 13 % of patients with chronic C with autoimmune features in accordance to our results which revealed positivity rates of 52.38% vs. 24.56% respectively. They found that all HCV patients with positive atypical p-ANCA showed ASMA with actin specificity, 10/12 (83%) of our anti-actin positive patients were also positive for atypical p-ANCA. Using autoantibodies in different combinations revealed that the best results were obtained when combining Homogeneous ANA, anti-actin SMA and p –ANCA with sensitivity 85.71%, specificity 73.68%, and Accuracy 76.92%.

Hypergammaglobulinemia was present in 100% of HCV/AIH overlap syndrome patients and in non of chronic HCV patients ( $p < 0.001$ ). Possible explanation of the above data was that since AIH is characterized by the presence of many kinds of autoantibodies the majority of them are of class IgG which result in elevation of  $\gamma$ -globulin concentration in patients sera while, HCV infection characterized by presence of one or 2 types of autoantibodies not enough to elevate IgG as high as in AIH. Hypergammaglobulinemia is well accepted to be a distinct feature of AIH, thus our result is in agreement with many investigators who collectively cited that serum level of  $\gamma$ -globulin rises in patients with AIH in comparison to HCV infection and healthy controls [25,26].

The mechanism by which, chronic HCV triggers autoantibodies (e.g. ANA and ASMA), is unclear. The virus may facilitate the expression of immunological abnormalities by stimulating the production of endogenous interferon [26]. Additionally, it may down regulate cell mediated immunity, exaggerating the humoral immunity with increased production of soluble CD23, which inhibits B cells apoptosis. CD23 has been found to be

elevated in; chronic HCV patients, systemic lupus erythematosus, Sjogren's syndrome, and rheumatoid arthritis patients [27, 28]. It is possible that this immune dysregulation seen in patients with chronic hepatitis C (CHC) may be compounded by the use of interferon  $\alpha$  in treatment of hepatitis C patients [29].

With the availability of highly sensitive assay techniques, some chemical tests become standard laboratory procedures in clinical practice for diagnostic and prognostic purposes. Therefore, in the present study biochemical parameters including serum AST, ALT, and alkaline phosphates were selected, there was significant association of aminotransferase with AIH, since this study showed that the highest concentration of aminotransferase AST and ALT were observed among patients with HCV/AIH overlap syndrome in comparison to those with HCV infection, this is probably due to the fact that AIH are aggressive form of the disease since the level of serum aminotransferase reflect severity of disease.

Histologic diagnosis of the overlap syndrome is another important consideration. Although no single histologic feature is pathognomonic of either HCV or AIH, distinct composite histologic patterns have been described for each entity. In general, patients with AIH are more likely to have severe lobular necrosis and inflammation, piecemeal necrosis, multinucleated hepatocytes, and broad areas of parenchymal collapse, whereas patients with HCV are more likely to have bile duct damage, bile duct loss, steatosis, and lymphoid cell follicles within portal tracts [30]. The combination of portal lymphoid aggregates and steatosis was found to have 91% specificity for HCV, whereas the pattern of lymphoplasmacytic portal, interface, and acinar hepatitis had 81% specificity for AIH [31]. In accordance with these findings, our results revealed that moderate to severe plasma cell infiltration of the portal tracts, Interface hepatitis and Rosettes were more common in patients with HCV/AIH overlap syndrome while, Portal-periportal necroinflammation and Lobular necroinflammation were more common in patients with chronic HCV infection.

To date, there are no standard guidelines on how to approach patients with HCV/AIH overlap syndrome [23]. One management strategy is to determine the predominant entity in order to select the appropriate type of therapy [19,32]. Chronic HCV-AIH overlap syndrome can be divided into autoimmune- or viral-predominant disease. Patients with autoimmune-predominant disease have ASMA or ANA titers of equal to or more than 1:320 or have ASMA and ANA titers of equal to or more than 1:40, as well as histology findings that include piecemeal necrosis (interface hepatitis), lobular hepatitis, and portal

plasma cell infiltrates. Patients with viral-predominant disease have ASMA or ANA titers of less than 1:320 or have antibodies to LKM type-1 and hepatitis C viremia, as well as histologic findings that include portal lymphoid aggregates, steatosis or bile duct injury. Tissue damage is more focal in HCV and more diffuse in AIH liver histology [33,34].

##### 5. Conclusions:

In chronic hepatitis C, serum autoantibodies are common, but their sub specificities are distinct from those occurring in AIH. Elevated  $\gamma$ -globulins is the best single test for selecting HCV/AIH overlap syndrome patients out of chronic HCV patients, followed by anti-actin, atypical pANCA, ANA, and the homogeneous ANA pattern, the worse was ASMA. Using antibodies in different combinations revealed that the best results were obtained when combining 3 antibodies: Homogeneous ANA, anti-actin SMA and p-ANCA. The absence of elevated  $\gamma$ -globulin levels, the low autoantibodies titre, absence of both anti-actin and the homogeneous ANA pattern should favour diagnosis of chronic viral hepatitis. It is recommended that: if the diagnosis HCV/AIH overlap syndrome is suspected these auto-antibodies should be looked at before the type of treatment is determined. Corticosteroids and/or immunosuppressive treatment can be the frontline therapy for patients with the overlap syndrome with predominant immunological features of AIH. IFN can be used as frontline therapy for patients with the overlap syndrome when the immunological features are more consistent with chronic HCV.

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## Study effect of salinity on some physiologic and morphologic properties of two grape cultivars

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**Abstract:** Salinity is a phenomenon challenging the plantation and growth of grape in arid and semiarid regions. During the present research, tolerance of two grape cultivars (Soltanin and Fakhri) was evaluated against various sodium chloride salinity levels (zero, 50, 100, 150, 200 and 250 mM), which was conducted based on factorial experiment in the form of Randomized Complete Design (RCD) with three replications at Agricultural and Natural Resource Researches Center (ANRRC) of East Azerbaijan, during 2011. Based on the obtained results, the cultivar and salinity levels were significantly effective on morphological and physiological traits. Moreover, the results from analysis of variance revealed the significant effects of salinity levels on rates of chlorophyll a and b; rate of chlorophyll a + b; photosynthesis and transpiration rate; stomatal conductance; dry weight of stem and root; concentrations of elements such as nitrogen, phosphorus, potassium, Chloride; plant height; leaf area; and relative water content (RWC). Furthermore, increased salinity levels led to significant decrease in values of majority of the abovementioned parameters. In contrast, the proline content, sodium and chloride concentrations increased as a result of increasing salinity. In addition, "Salinity × cultivar" interaction also proved significantly effective on traits such as plant height, leaf area, dry weight of stem, proline content, chlorophyll a and b, chlorophyll a + b, photosynthesis and transpiration rate, stomatal conductance, dry and fresh weights of stem and root, nitrogen and sodium content of leaf and RWC. More specifically, the lowest values for the abovementioned parameters were measured at 250 mM sodium chloride salinity level for Fakhri cultivar. Without salinity application Soltanin produced the best values for physiological and morphological indices. In general, Soltanin cultivar proved more tolerant against salinity than Fakhri cultivar did.

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**Keywords:** *Vitis vinifera* L., cultivar, salinity

### Introduction:

Salinity or increased concentration of soluble salts in cultivated soils is one of the main challenges for sustainable agriculture, with a decreasing effect on plant growth and specifically on horticultural crops yield. Roughly 15% of lands in East Azerbaijan are considered as saline and brackish and are threatened by salinity. Unless their productivity and crop yield from them do not meet the economical expectations, cultivation in these lands should not be ignored due to limiting effect of salinity on plant growth. Undoubtedly, any research addressing the effect of salinity on the physiological characteristics of the grape will contribute greatly to a rational advance in grape production. As the salinity in most of the fertile lands such as regions surrounding the Orumiyeh Lake is affected by sodium chloride, this experiment will be able to identify salinity tolerance rate for widely cultivated native grape species within the prospect to expand the

planting area of grape in the province. Furthermore, in addition to fresh consumption and raisin production from grape, its leaves also are used to prepare various Dolmas (foods). As the mineral content of grape leaves is important for a human and plant balanced nutrition, it will prove highly useful to investigate the effect of various salinity levels on chemical composition of grape leaves also (1).

In saline soils, concentrations of sodium and chloride in soil solution is generally higher than that of most of the other elements and it not only causes osmotic stress and specific ion effect, also leads to disorder in uptake of other elements as well as their translocation into aerial organs of the plant (nutritional diseases) and consequently decreases plant yield (2 and 3). The effects of salinity on both quantity and quality of grape have been researched in multitudes of investigations conducted in and out of the country. Salinity tolerance threshold for this plant reportedly is 1.5 dS.m<sup>-1</sup>, while at 2.5

dS.m<sup>-1</sup> the plant growth decreases by 10% (4 and 5). However, it's worth consideration that cultivars of the species of a given plant vary greatly in terms of their tolerance against salinity.

The first response of glycophytes plants in face of salinity stress is decreased and finally ceased growth of their leaves (name, year). Gomez et al. (2002) demonstrated that decreased total leaf area was the result of decreased number and size of the leaves, as such that the numbers of leaves were decreased to 11 (at 50 mM concentration) and 18 (at 100 mM), whereas their size decreased 29% (at 50 mM) and 46% (at 100 mM), respectively. Moreover, in this research the salinity decreased the dry plant weight through decreasing branch length and total leaf area. Interestingly, the salinity affected more leaf area than branch length.

Biomass of Asghari and Soltanin cultivars were decreased by 34 and 64% at 50 and 10 mM NaCl concentrations, respectively. At lower salinity level (25 mM), decreased percentage of dry weight of aerial organs, which was similar to decreased percentage of dry weight of roots, which suggests that branches and roots are equally susceptible against NaCl salinity. Growth of root was less affected by salinity at 50, particularly 100 concentrations, than that of branches (name year). Singh (year) demonstrated that increasing salinity level led to decreased dry and fresh weights of aerial organs as well as number of stomatas and node interval.

Estion and Harvey (35) conducted an in vitro experiment in order to determine the salinity tolerance in some grape cultivars and demonstrated that salinity tolerant cultivars maintain their growth rate to a relative extent, and are capable of dealing with metabolic disorders such as chlorophyll deficiency. Salinity experiments on cultivars such as Chavosh, Moshkeleh and Soltanin was conducted under laboratorial conditions and by using lateral seedling planting method. During the experiment, germination, growth, chlorophyll content and healthy of vegetative samples decreased as a result of increased concentration of NaCl and extended period of the treatment. In addition, it was found that salinity treatment caused various rate of necrosis in the samples dependent on the cultivar, NaCl concentration and treatment period. In general, Chavosh had the highest tolerance against NaCl salinity, followed by Soltanin and then by Moshkeleh.

Salinity tolerance in fruit trees, particularly in grape tree, is heavily influenced by cultivar. Results from the research revealed that the capacity of cultivars to regulate the absorption of Na<sup>+</sup> and Cl<sup>-</sup> determines their tolerance, i.e. the higher the capacity of plant in preventing the uptake of Na<sup>+</sup> and Cl<sup>-</sup>, the higher will be its tolerance. Salinity stress produces both short-term and long-term effects. One or two days after the plant exposure to salinity, it takes only a few hours for the short-term effects to take place, during which a complete cessation of carbon assimilation is resulted. Whereas, the long-term effects after the exposure of plant to salinity for several days and decreased carbon assimilation, happens due to salt accumulation in the leaves (15).

Study by Flexas et al. (19) on the response of 6 cultivars of grapevine against sodium chloride salinity revealed that increasing salinity significantly increased the salt content of plant tissue. Curiously, this research investigated plant response with respect to growth, minerals content of tissues as well as gas trade-offs, and corresponding results showed that growth, dry matters of aerial organs, leaf area and total dry weight decreased significantly at all salinity levels. Growth measurement was achieved through measuring leaf area, leaf number, dry and fresh weights of plant and root; as well as through the measurement of gas trade-offs including leaf photosynthesis, stomatal conductance, respiration rate and intracellular concentration of CO<sub>2</sub>.

Grapevine trees are relatively susceptible against salinity (18) and the main damages are caused by chloride ions (20). Mass (26) estimated the tolerable chloride concentration for Dogrils and Salt creek cultivars of grape to be 80 and 60 M/m<sup>3</sup>, respectively. It is well established that grapevine response against salinity depends upon various factors such as combination of rootstock and scion, cultivar, irrigation system, type of soil and climate. Shani and Ben-Gal (28) believed that growth decline as a result of salinity is often attributed to such factors as ion toxicity and/or low osmotic potential, which are capable of influencing physiological and biochemical processes. For instance, study by Walker et al. (36) on Soltanin cultivar suggests that photosynthesis and stomatal conductance are heavily influenced by sodium chloride salinity and are directly connected to ratio of high concentration of Cl<sup>-</sup> to Na<sup>+</sup> in the leaves. It's worth mentioning that grapevine cultivars vary in their tolerance against salinity.

This research focuses on evaluating the tolerance of two grape cultivars against various salinity levels.

### Materials and methods:

In order to investigate salinity effect on two widely cultivated grape cultivars in East Azerbaijan Province, a factorial experiment based on RCD, with three replications, was conducted at greenhouse of department of soil and water researches of ANRRC, in 2011. First factor was sodium chloride salinity in six levels (zero, 50, 100, 150, 200 and 250 mM), whereas the second factor included two grape cultivars namely Soltanin and Fakhri in four replications. Grape scions were planted in beds containing equal proportions of sand, perlite and vermiculite in 20-L vases. Each vase was supported on a saucer and the electric conductivity (EC) of both incoming and outgoing solutions was controlled. During the first two to three weeks after the plantation, the vases were nourished by half Hoagland nutrient solution. In order to create the desired salinity levels, another half of the Hoagland nutrient solution was salinized with 0, 50, 100, 150, 200 and 250 mM concentrations of sodium chloride and their ECs were measured. After the establishment of scions, the vases were irrigated by the prepared solutions. In addition to EC, pH of leaching water of the vases was measured throughout the growth period. In case the salinity of leaching water was in excess of the salinity levels determined for this study, the beds were irrigated and leached by tap water. Throughout the growth period, measurement was done on growth parameters such as plant height, leaf area, dry and fresh weights of aerial organs and roots. In addition, the chlorophyll index of leaves was measured for at least three stages of plant growth by using chlorophyll Meter (SPAD-504). Concentrations of elements such as N, P, K, Cl<sup>-</sup>, and Na were measured in leaf throughout the growth season, and in aerial organs and roots after the harvest. Moreover, the photosynthesis was measured using Photosynthesis Meter (Model Da-1000, Wallz Co., Germany), and last but not least, the measurement was conducted

between 9 A.M and 2 P.M local time under a fixed light intensity.

Chlorophyll a and b was measured using Arnon method. In this method, as little as a half gram of wet vegetative matter was chopped and thoroughly mashed in liquid nitrogen, in a porcelain mortar. As much as 20mL of 80% acetone was added to the sample, and then the mixture was put into centrifuge device with 6000 rpm speed for 10 minutes. Supernatant was transferred into a glass balloon. Some of the samples in the balloon were read in spectrophotometer for chlorophyll a at 663nm; for chlorophyll b at 645nm; and for Carotenoids at 470nm. Finally, the following formulas were used to calculate chlorophyll a and b and carotenoids contents in mg/g of fresh weight of the sample.

$$\text{Chlorophyll a} = (19.3 * A_{663} - 0.86 * A_{645}) V/100W$$

$$\text{Chlorophyll b} = (19.3 * A_{645} - 3.6 * A_{663}) V/100W$$

Stem height was measured by a ruler. The leaves were counted and then towards the end of the experiment Leaf Area Meter (Model 200) was used to measure area of the leaves. Using a 0.0001 scale, dry and fresh weight of leaves and dry weight of stem and root were measured. In order to determine the dry weight, prior to weighing the samples were put in 70°C for 72 hours to achieve the desired desiccation.

Moreover, in order to estimate relative water content (RWC) of the leaves, two completely developed leaves were cut and removed from each of plants and 10 leaf disks, 8 mm in diameter each, was cut from the middle part of the blades. The disks were weighed and then put into lidded petri dishes containing distilled water and kept in refrigerator at 4°C under dark conditions for 24 hours. After removing the disks from distilled water, they were blot dried to remove the excessive humidity; then their water saturated weight was measured. Then, they were transferred to a 70°C for 48 hours before being weighed for their dry weights. The following equation was used to obtain RWC of the leaves.

$$\text{RWC} = \frac{\text{fresh weight of the leaf disks} - \text{dry weight of the leaf disks}}{\text{inflated weight of the leaf disks} - \text{dry weight of the leaf disks}} \times 100$$

Temperature of the leaf was read and recorded using infrared thermometer (Hi 99550 Hana) from a distance as far as 4 cm from two

randomly selected vases from each unit. Chlorophyll indices of the leaves were measured by using Chlorophyll Meter (SPAD – 502 –

Minolta Osaka Model, Japan), whereas Paquin and Lechasseur (1976) method was used to measure the proline content.

As for measuring the proline density, 1 mL of the prepared alcoholic extract was diluted by 10 mL of distilled water before applying 5 mL of Ninihydrin as reagent (the preparation method of Ninihydrin for each sample was: 0.125 g ninihydrin + 2 mL phosphoric acid 6 molar + 3 mL of glacial acetic acid). Furthermore, the application of reagent ninihydrin was followed by adding as much as 5 mL of glacial acid acetic to the solutions, which were stirred for 45 minutes in boiling water bath and cooled off before applying 10 mL of benzene for each samples (32 samples) and then were stirred so intensively that the proline entered benzene phase. Then the samples were left immobile for 30 minutes. Some standards of proline were prepared with density ranging from zero through 0.1 mM/mL (0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09 and 0.1 mM/mL) and finally the absorption rate of standard solutions and of samples were measured at 515 nm by using spectrophotometer. Data was analyzed using MSTATC software, whereas diagrams were drawn by Excel software.

## Results

Based on results from Table of analysis of variance, the salinity levels had a significant effect on traits such as plant height; leaf area; fresh stem weight; proline content; chlorophyll a; chlorophyll b; chlorophyll a + b; photosynthesis and transpiration rate and stomatal conductance; fresh and dry weight of root; dry weight of stem; nitrogen, phosphorus, potassium, chloride, and sodium content of leaf; and RWC (Table 1).

Interaction of "salinity × cultivar" was found to be significant on traits such as fresh stem weight, proline content, chlorophyll a, chlorophyll b, and dry stem weight. The highest plant height (19.5 cm) was produced at control treatment, whereas the lowest value (8.45 cm) for this trait was measured at salinity level of 250 mM. Likewise, the highest leaf area (96.3 cm<sup>2</sup>) was measured at control treatment, whereas the lowest value for this trait was produced at 250 mM sodium chloride treatment. Moreover, the increasing salinity levels had a significantly decreasing effect on fresh and dry weights of both root and stem, while the lowest values for these traits were produced in 250 mM sodium level. In contrast, the increasing salinity caused a

significant increase in proline content of grape leaf, while the highest value (35.5 mg/g) was measured in 250 mM treatment (Table 2).

Moreover, increasing salinity level had a significantly decreasing effect on contents of chlorophyll a, b and of chlorophyll a + b, while the lowest values were found at 250 mM sodium chloride level. Likewise, photosynthesis and transpiration rate and stomatal conductance declined significantly in the face of increasing salinity levels, while the lowest values for these parameters were obtained at 250 mM sodium chloride salinity level. Furthermore, increasing salinity level led to a significant decrease of nitrogen, phosphorus and potassium content of leaf. Conversely, it led to a remarkable increase in the sodium and chloride content of the leaf. As for RWC, the highest value (88.78%) was produced at without salinity application treatment (control), whereas the lowest (36%) at application of 250 mM sodium chloride (Table 2).

Soltanin cultivar exhibited more efficiency with respect to quantitative factors such as plant height, leaf area, fresh and dry weights of both stem and root than Fakhri cultivar (Table 2). Similarly, highest values for traits such as Proline content, content of chlorophyll a, b and of chlorophyll a + b, photosynthesis and transpiration rate, stomatal conductance and RWC were more in Soltanin cultivar than in Fakhri cultivar. In addition, Soltanin cultivar had the highest concentration of nitrogen, phosphorus and potassium, whereas Fakhri had the highest content of sodium and chloride.

The highest plant height (21 cm) and highest fresh stem weight (85 gr) belonged to Soltanin cultivar in control, whereas Fakhri produced the lowest plant height (6.5 cm) and lowest fresh stem weight (28 g) in 250 mM sodium chloride treatment. As for dry stem weight, the highest (21 g) and lowest (6.5 g) values were obtained in control and 250 mM sodium chloride treatment, respectively, while both belonged to Fakhri cultivar. Soltanin cultivar was able to produce the highest (35 mg/g) proline content in 250 mM sodium chloride treatment. It also produced the highest contents of chlorophyll a (6 mg/g) and chlorophyll b (3.8 mg/g) in the leaf in control, which decreased with the increasing salinity level. Furthermore, the highest rate of photosynthesis (9.5  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ ) happened in control in Soltanin cultivar, whereas the lowest rate (5.2  $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ ) happened in 250 mM sodium chloride treatment and in Fakhri cultivar.



## Discussion

Presence excess salt in planting medium was found to be one of the main reasons for decreased number of root, while extreme condition such as a concentration as high as 200 to 250 mM of sodium chloride in the planting medium practically stopped root development. In addition, increased osmotic pressure in the medium combined with the increasing salt presence had a decreasing effect on root development, which subsequently delayed their appearance. Furthermore, increased salt concentration in the medium would lead to more negativity of already negative osmotic pressure in the root growth zone as well as toxic effect of high salt concentration, which would not create a favorable condition for root growth. Interestingly, the increased salt concentration in the environment not only negatively influenced root development, but also stem development.

Authors believe that total decrease in fresh and dry weights of stem may not relate to efficiency of leaf area to produce photosynthetic substances, rather to decreased number of leaves, or more specifically to decreased leaf area. The change in plants as a result of increased salt may reveal as plant's failure to take up more ions under salinity stress condition or failure in quick translocation of ions to leaves and their distribution in the leaf cells. During low concentrations of salt, as the uptake or translocation of the ions are characteristically selective, root begins to take up sodium ion in consistence with increasing salinity; however in high salinity levels this mechanism fails as there is no practical root development. Consequently, grape is classified among the salt-absorbing and salt-storing plants in the face of increasing salts in its planting medium (17).

Grape cultivars differed in their response against various salinity levels. In saline soils, vegetative growth and leaf development are influenced in the first place. Furthermore, the decreased growth and development of plants in saline soils is linked with increased osmotic pressure associated with presence of sodium, chloride, magnesium and sulfate ions, which ultimately makes water less usable by the plant (16).

One of the remarkable effects of salinity is declined vegetative features such as plant height and leaf area of grape. Majority of the authors have related such a decline to mitigated photosynthesis due to increased salinity levels (150-200 mM). In this study, also the decreased chlorophyll index as well as decreased rate of

chlorophyll a and b as a result of increased salinity level has led to decreased dry weights of leaf, stem and roots of cultivars. Under stress condition, there is a competition between aerial organs and root in uptake of photosynthetic substances, which negatively influence these organs (15).

In this experiment, increasing salinity level had a decreasing effect on RWC of the leaves. This may be accounted for by status of stomatas and increased transpiration rate of the leaves. Osmotic regulation is an indication of response to osmotic stress and when there is a water limitation caused by salinity stress, osmotic potential is declined and this in turn causes the reduction of RWC of the leaves.

Osmotic regulation depend upon the cultivar as well as on decreased rate of water potential and this is safe to say that one of the mechanisms of tolerance against salinity in grape is to maintain high RWC of the leaf. RWC is mainly positively correlated with leaf area, dry leaf weight, chlorophyll content and other growth indices. Furthermore, increasing salinity level had a decreasing effect on chlorophyll content of the leaf, while this was more evident in the leaves of grape soltani than in cultivar fakhri. In contrast, increasing salinity stress had a significantly increasing effect on proline content of the leaves, while this was more evident in cultivar fakhri than cultivar soltani. It is known that salinity stress reduces chlorophyll content, because the glutamate which is the primary constituents of chlorophyll and proline is consumed in favor of proline production. Furthermore, salinity stress induce glutamate ligase enzyme to transform glutamate into proline. Another reason for chlorophyll reduction is the increased use of nitrogen for proline synthesis. Proline plays a key part in maintaining the osmotic pressure and cytoplasmic enzymes and protects cell membrane from any damage through absorbing free radicals. Different researchers also believe that decreased chlorophyll content may be due to inhibitory effect of ions accumulated in chloroplast, chlorophyll degradation by oxidative stress caused by salt, activation of chlorophylase enzyme by salinity ions and its negative effect on protophyzine. Increasing salinity level leads to decreased chlorophyll biosynthesis through increased salt. It causes a rise in leaf temperature and consequently the stomatas are closed due to water limitation stress caused by salinity, at the same time due to synthesis of abscisic acid in the root and its translocation to the stomatas. In addition, shrinking of the mezophyllic cells

contribute to synthesis of abscisic acid and its translocation to stomatal cells. Decreased stomatal conductance as a result of this phenomenon leads to a rise in leaf temperature, because, as a rule the leaves get rid of the excessive heat through doing transpiration.

### Conclusion

Results from the study revealed that Soltani cultivar was more tolerant against salinity than Fakhri cultivar, because Soltani produced higher values for the majority of morphological indices such as plant height, leaf area, dry and fresh weights of stem and root than Fakhri did. In addition, other mechanisms including RWC and proline concentration makes it a tolerant cultivar for overcoming salinity stress, whereas Fakhri could not potentially employ this mechanism as efficiently as Soltani could, due to lower accumulation of proline.

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**Table 1. Analysis of variance on different characteristic of grape affected by samity and cultivars.**

Sources of variation	Degree of freedom	Height	Leaf area	Stem F. W	Proline	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Raet of photosynthesis	Transpiration rate	Stomatal conductance	Stem D. W	N(%)	P(%)	K(%)	Cl (mg.g <sup>-1</sup> )	Na (mg.g <sup>-1</sup> )	RWC(%)
Salinity	5	100.85***	2940.03***	1293.15***	354.03***	9.19**	4.02**	25.30***	27.98**	14.80***	0.03**	108.66***	3.02**	0.018**	1.45***	0.97 <sup>ns</sup>	205.41	2405.81***
Cultivars	1	31.37***	1681***	930.86***	930.86 <sup>ns</sup>	2.83**	2.28 <sup>ns</sup>	10.24***	2.20**	9.20***	0.01**	46.58**	0.284 <sup>ns</sup>	0.027***	0.16 <sup>ns</sup>	0.52 <sup>ns</sup>	110.25	171.61***
Salinity x cultivars	5	2.29 <sup>ns</sup>	26.51 <sup>ns</sup>	303.50**	330.52***	5.40**	6.10**	0.82	0.48 <sup>ns</sup>	0.71 <sup>ns</sup>	0.001 <sup>ns</sup>	88.60**	0.035 <sup>ns</sup>	0.001 <sup>ns</sup>	0.035 <sup>ns</sup>	0.054 <sup>ns</sup>	32.25	8.38 <sup>ns</sup>
Error	24	3.74	254.79	187.99	43.41	0.24	0.27	0.78	0.98	1.62	0.001	5.37	0.139	0.002	0.08	2.02	16.01	38.97
C.V (%)	-	14.71	24.79	18.99	16.41	14.21	15.20	17.45	13.52	8.62	18.25	15.37	10.12	9.32	17.41	23.02	16.01	25.97

\*, \*\*, ns: significant at 0.05, 0.01 probability level and no significant

**Table 2. Main effect salinity on different characteristics of grape.**

parameter Salinity Levels	Height (cm)	Leaf area (cm <sup>2</sup> )	Stem F. W (gr)	Praline	Dry stem weight (gr)	Photosynthesis ( $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ )	Transpiration rate( $\mu\text{mol.m}^{-2}.\text{s}^{-1}$ )	Stomatal conductance	RWC(%)	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	N (%)	P (%)	K (%)	Na (mg- 1g)	CL (mg- 1g)
0NaCl	19.50 <sup>a</sup>	96.33 <sup>a</sup>	74.04 <sup>a</sup>	15.41 <sup>f</sup>	18.47 <sup>a</sup>	9.23 <sup>a</sup>	6.65 <sup>a</sup>	0.36 <sup>d</sup>	88 <sup>a</sup>	5.57 <sup>a</sup>	3.70 <sup>a</sup>	9.27 <sup>a</sup>	4.13 <sup>a</sup>	0.30 <sup>a</sup>	3.61 <sup>a</sup>	4.56 <sup>f</sup>	1.58 <sup>b</sup>
50 mM	16.71 <sup>b</sup>	79.68 <sup>b</sup>	62.08 <sup>b</sup>	18.01 <sup>f</sup>	14.90 <sup>b</sup>	7.75 <sup>b</sup>	5.55 <sup>b</sup>	0.28 <sup>b</sup>	72 <sup>b</sup>	4.57 <sup>b</sup>	3.13 <sup>a</sup>	7.70 <sup>b</sup>	3.60 <sup>b</sup>	0.22 <sup>a</sup>	3.28 <sup>a</sup>	7.58 <sup>e</sup>	2.01 <sup>ab</sup>
100	14.61 <sup>c</sup>	67.81 <sup>c</sup>	53.92 <sup>c</sup>	22.32 <sup>f</sup>	11.33 <sup>c</sup>	6.65 <sup>c</sup>	4.52 <sup>c</sup>	0.27 <sup>b</sup>	63 <sup>c</sup>	3.72 <sup>c</sup>	2.63 <sup>b</sup>	6.35 <sup>c</sup>	3.18 <sup>b</sup>	0.21 <sup>c</sup>	2.93 <sup>b</sup>	10.55 <sup>d</sup>	2.17 <sup>a</sup>
150	11.97 <sup>d</sup>	54.40 <sup>d</sup>	46.12 <sup>d</sup>	26.52 <sup>e</sup>	9.78 <sup>d</sup>	5.53 <sup>d</sup>	3.86 <sup>d</sup>	0.18 <sup>b</sup>	51 <sup>d</sup>	3.17 <sup>c</sup>	2.33 <sup>b</sup>	5.50 <sup>d</sup>	2.75 <sup>c</sup>	0.18 <sup>d</sup>	2.70 <sup>b</sup>	15.93 <sup>c</sup>	2.43 <sup>a</sup>
200	10.55 <sup>e</sup>	47.45 <sup>e</sup>	37.95 <sup>e</sup>	30.88 <sup>b</sup>	8.37 <sup>e</sup>	4.10 <sup>e</sup>	3.03 <sup>d</sup>	0.16 <sup>c</sup>	40 <sup>e</sup>	2.70 <sup>d</sup>	1.92 <sup>c</sup>	4.62 <sup>e</sup>	2.38 <sup>c</sup>	0.15 <sup>e</sup>	2.46 <sup>b</sup>	19.93 <sup>a</sup>	1.61 <sup>a</sup>
250	8.45 <sup>f</sup>	35.25 <sup>f</sup>	36.03 <sup>f</sup>	35.52 <sup>a</sup>	7.29 <sup>f</sup>	3.66 <sup>f</sup>	2.45 <sup>e</sup>	0.15 <sup>e</sup>	36 <sup>f</sup>	2.23 <sup>d</sup>	1.44 <sup>e</sup>	3.66 <sup>f</sup>	2.35 <sup>c</sup>	0.13 <sup>f</sup>	2.33 <sup>b</sup>	16.40 <sup>a</sup>	2.61 <sup>a</sup>

Values within the each column and followed by the same letter are not different at P<0.05 by an ANOVA protected Duncan's Multiple Range- Test

**Table 3 Main effect salinity and cultivars on different characteristics of grape.**

Parameters Salinity Levels		Height (cm)	Stem F. W (gr)	Stem F. W (gr)	Proline(mg- 1g)	Chlorophyll a	Chlorophyll b	Photosynthesis rate( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	Transpiration rate ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ )	RWC(%)
NaCl mM 0	Soltanin	21.50 <sup>a</sup>	75.40 <sup>a</sup>	21.50 <sup>a</sup>	16.20 <sup>f</sup>	6.10 <sup>a</sup>	4.80 <sup>a</sup>	9.8 <sup>a</sup>	0.49 <sup>a</sup>	98 <sup>a</sup>
	Fakhri	19.25 <sup>b</sup>	65.20 <sup>b</sup>	17.10 <sup>b</sup>	15.30 <sup>e</sup>	5.80 <sup>b</sup>	3.50 <sup>b</sup>	8.7 <sup>b</sup>	0.38 <sup>b</sup>	82 <sup>ab</sup>
50 mM	Soltanin	19.10 <sup>b</sup>	65.30 <sup>b</sup>	17.20 <sup>b</sup>	18.60 <sup>e</sup>	5.10 <sup>b</sup>	3.60 <sup>b</sup>	8.2 <sup>c</sup>	0.38 <sup>c</sup>	78 <sup>b</sup>
	Fakhri	15.20 <sup>c</sup>	56.20 <sup>c</sup>	14.15 <sup>c</sup>	17.20 <sup>f</sup>	4.60 <sup>c</sup>	3.80 <sup>c</sup>	6.8 <sup>d</sup>	0.29 <sup>c</sup>	61 <sup>b</sup>
100	Soltanin	15.20 <sup>c</sup>	55.20 <sup>c</sup>	11.60 <sup>d</sup>	26.60 <sup>d</sup>	4.0 <sup>c</sup>	3.20 <sup>c</sup>	7.20 <sup>d</sup>	0.30 <sup>c</sup>	69 <sup>bc</sup>
	Fakhri	13.30 <sup>d</sup>	46.10 <sup>d</sup>	11.20 <sup>e</sup>	24.40 <sup>e</sup>	3.40 <sup>d</sup>	2.70 <sup>d</sup>	6.2 <sup>e</sup>	0.26 <sup>d</sup>	56 <sup>d</sup>
150	Soltanin	12.40 <sup>d</sup>	48.60 <sup>d</sup>	9.60 <sup>e</sup>	28.40 <sup>e</sup>	3.10 <sup>d</sup>	2.60 <sup>d</sup>	5.1 <sup>f</sup>	0.26 <sup>d</sup>	58 <sup>e</sup>
	Fakhri	11.30 <sup>e</sup>	36.15 <sup>e</sup>	9.20 <sup>f</sup>	27.20 <sup>e</sup>	2.80 <sup>e</sup>	2.50 <sup>e</sup>	4.8 <sup>e</sup>	0.20 <sup>e</sup>	46 <sup>e</sup>
200	Soltanin	11.20 <sup>e</sup>	36.10 <sup>e</sup>	8.20 <sup>e</sup>	32.20 <sup>b</sup>	2.10 <sup>e</sup>	2.10 <sup>e</sup>	3.1 <sup>e</sup>	0.20 <sup>e</sup>	42 <sup>e</sup>
	Fakhri	10.60 <sup>e</sup>	35.10 <sup>f</sup>	7.40 <sup>e</sup>	31.10 <sup>b</sup>	1.90 <sup>f</sup>	2.00 <sup>f</sup>	2.8 <sup>h</sup>	0.16 <sup>f</sup>	34 <sup>f</sup>
250	Soltanin	6.80 <sup>f</sup>	26.80 <sup>f</sup>	7.10 <sup>e</sup>	36.75 <sup>a</sup>	1.90 <sup>f</sup>	1.40 <sup>f</sup>	1.1 <sup>h</sup>	0.18 <sup>f</sup>	30 <sup>e</sup>
	Fakhri	5.40 <sup>f</sup>	25.10 <sup>f</sup>	6.10 <sup>f</sup>	35.40 <sup>a</sup>	1.60 <sup>f</sup>	1.30 <sup>f</sup>	1.0 <sup>h</sup>	0.14 <sup>e</sup>	26 <sup>h</sup>

Values within the each column and followed by the same letter are not different at P<0.05 by an ANOVA protected Duncan's Multiple Range- Test

## Is Lysosomal Enzymes Changes Important In the Pathogenesis of Liver And Kidney Injury Induced By Short and Long Term Administration of Some NSAID' Drugs in Rats?

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**Abstract:** Moderate lysosomal membrane permeabilization is an important inducer of apoptosis. The objective of this study was to investigate the harmful hepatotoxicity and nephrotoxicity effects induced by short and long term administration of paracetamol, nimesulide, and lornoxicam drugs in rats. Results revealed that liver and kidney lysosomal enzymes activities (ACP,  $\beta$ -NAG and  $\beta$ -GAL) were significantly increased by paracetamol followed by nimesulide in acute study as compared with chronic study, while no changes were observed in lysosomal enzymes of lornoxicam group in both studies. Serum liver enzymes activities (AST, ALT and  $\gamma$ -GT) and (urea and creatinine) levels were significantly increased by paracetamol followed by nimesulide and lornoxicam in chronic study as compared with acute study. Liver and kidney GSH levels and antioxidant enzymes activities were significantly decreased by paracetamol followed by nimesulide in acute study as compared with chronic study, while no changes were observed by lornoxicam in both organs either in acute or chronic studies. Liver and kidney MDA levels were significantly increased by paracetamol followed by nimesulide in acute study as compared to chronic study. These results demonstrated that liver and kidney functions were affected by oxidative stress greatly by paracetamol than nimesulide or lornoxicam in both studies.

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**Key words:** paracetamol; nimesulide; lornoxicam; Hepatotoxicity; nephrotoxicity; lysosomal enzymes; antioxidant enzymes.

### 1. Introduction

Hepatotoxicity is the potential complication of most drug therapies prevalent in patients treated with nonsteroidal anti-inflammatory drugs (NSAIDs) (Bjornsson, 2010). NSAIDs are the most frequently prescribed therapeutic agents. Due to its over use, pronounced side effects occur, which range from mild, transient elevations in serum transaminases to pronounced hepatocellular and/or cholestatic injury sometimes leading to fatal fulminant hepatitis. Cases of reported hepatotoxicity are one of the important reasons resulting in withdrawal of some of the popular NSAIDs (Han *et al.*, 2010).

Paracetamol is a widely used analgesic and antipyretic drug and is safe at therapeutic doses but accidental or intentional overdose causes acute liver and kidney failure (Boutis and Shannon, 2001 and Larson *et al.*, 2005). Because of its clinical importance Paracetamol -induced acute toxicity has become an indispensable model for studying drug-induced liver and kidney injury. At therapeutic doses, PAR is metabolized via glucuronidation and sulfuration reactions occurring primarily in the liver, and results in water-soluble metabolites that are excreted via the kidney.

Nimesulide (N-(4-nitro-2-phenoxyphenyl) methanesulfonamide), an NSAID with anti-inflammatory, anti-pyretic and analgesic properties, is one of the most prescribed drug with largest market as compared to other NSAIDs (Kalra *et al.*, 2009). Reactive oxygen species (ROS) and resultant mitochondrial dysfunction has been implicated as a general mechanism in the toxicity of many NSAIDs (Han *et al.*, 2010). Several groups have suggested that intracellular ROS generation may also constitute a conserved apoptotic event and cite ROS production as a critical determinant of toxicity associated with exposure to such drugs (Zhang *et al.*, 2010). As mitochondria are the major site of ROS production, hence they may create adequate oxidative stress which may lead to organelle dysfunction, ultimately leading to cell death. Mitochondria have also been found to be frequently involved in the toxicity of many drugs and other xenobiotics and have been the subject of excellent reviews (Han *et al.*, 2010).

Lornoxicam, a new oxycam derivative, is a strong anti-inflammatory agent. Studies have shown that lornoxicam inhibits polymorphonuclear (PMN) leukocyte migration, superoxide release from human PMN leukocytes and nitric oxide release from

macrophages (Pruss *et al.*, 1990; Berg *et al.*, 1999 and Radhofer-Welte and Rabasseda, 2000).

In chronic health problems where use of NSAIDs becomes imperative, it is logical to search for alternative/complimentary medicines which can reduce its toxicity. In cases of liver injury, antioxidants hold promise.

The aim of the present study was undertaken to investigate the comparative biochemical changes in liver and kidney of rats induced by NSAIDs *in vivo*, and also to identify the drugs most commonly responsible for lysosomal enzymes dysfunctions *in vitro*.

## 2. Materials and Methods

### Animals

The animal studies were carried out in compliance with policies outlined in the 'Guide for the Care and Use of Laboratory Animals', published by the US National Institute of Health (NIH Publication No. 85-23, revised 1996). Experiments were carried out on adult male Albino rats aged 10-12 weeks and weighing 150-200 g obtained from the animal house of the National Research Centre (Giza, Egypt). Rats were housed in groups of five per cage under controlled environmental conditions of temperature and humidity and exposed to a 12-h light/dark cycle. Unless otherwise indicated, animals were fed on normal laboratory chow and given tap water *ad libitum* throughout the experimental work.

### Experimental protocol

Animals were divided into two main groups acute (4 days) and chronic (4 weeks), then, each main group was further subdivided into 4 subgroups as follows: I) Normal control (C) group: received 1% tween 80 in distilled water as a vehicle. II) Paracetamol-treated group (PAR): received paracetamol in a dose of 500mg/kg b.wt./day (Garba *et al.*, 2009). III) Nimesulide- treated group (NIM): received nimesulide in a dose of 18mg/kg b.wt./day (Singh *et al.*, 2003). IV) Lornoxicam- treated group (LOR): received lornoxicam in a dose of 1.4mg/kg/day (Ayan *et al.*, 2008). All groups were administered the different drugs for four days (acute study) and four weeks for the (chronic) one.

### Drugs and Chemicals

Drugs: Paracetamol (N-acetyl p-amino phenol) obtained from Amiriya pharmaceutical industries; nimesulide (4-Nitro-2-phenoxy methane sulphonanilide) obtained from Sigma pharmaceutical industries; lornoxicam (Chlorotenoxicam) obtained from Delta pharmaceutical industries, Egypt. All chemicals for which source is not noted were of analytical grade from Sigma, Fluka, BDH, Park, and Riedel-de Haën.

## Assays

At the end of the experimental period, the animals were fasted overnight and were then sacrificed by cervical decapitation (between 9:00 and 11:00 am), 24 h after the last dose application. Blood samples were obtained from the Retro-orbital venous plexus (by means of fine capillary glass tube), in clean and dry heparinized centrifuge tubes (Becton Dickinson Co., Rutherford, NJ) and centrifuged (800 x g, 15 min, 4 °C). The separated plasma was used for the colorimetric assay of Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities (Reitman and Frankel, 1957), while plasma  $\gamma$ -glutamyl aminotransferase ( $\gamma$ -GT) was determined by kinetic colorimetric method according to Szasz and Persijn (1974). Plasma urea and creatinine concentrations were measured according to Mackey and Mackay (1982) and Bartles and Bohmer (1972) respectively.

Immediately after decapitation, rats were dissected for isolation of liver and both kidneys. The separated liver and kidneys were rinsed with ice-cold saline, blotted dry, weighed, and homogenized in ice cold normal saline using a Potter Elvehjem Homogenizer fitted with a Teflon plunger. Homogenates were sonicated at a low frequency (10 kHz), centrifuged at 3,000 rpm for 15 minutes at 4°C to remove the nuclear debris using (Sigma Lab. Centrifuge 3K30), fractions of the resultant supernatant were used for the assays of reduced glutathione (GSH) according to the method of Ellman (1979) as modified by Ahmed *et al.* (1991), malondialdehyde (MDA) by the method of Buege and Aust (1978), the activity of catalase (CAT) was determined by the method of Takahara *et al.* (1960) and protein content (Lowry *et al.*, 1951).

Cytosolic fractions of liver and kidney are prepared by centrifuging homogenates (30,000 r.p.m, 15 min, 4°C), using (Sigma Lab. Centrifuge 3K30) and the cytosolic fractions were separated and used for superoxide dismutase (SOD) determination (Marklund and Marklund, 1974).

### Preparation of liver lysosomal fraction:

Liver lysosomal fraction was prepared according to the method of Tanaka and Iisuka (1968). 1 gm of liver tissue was homogenized in 3 ml of 0.25 M sucrose buffer (pH 7.4). After homogenization the volume were adjusted to 6 ml with sucrose buffer. The homogenates were then centrifuged (820 xg, 15 min, 4°C). The supernatant was separated and the sediment washed and recentrifuged under the same condition, then the supernatant was separated and added to the first supernatant. The whole lysosomal fractions were prepared by centrifuging the combined supernatant (14,000 xg, 15 min, 4°C). The sediment was washed and resuspended in 0.25 M sucrose



buffer and this step was repeated three times for isolating pure lysosomal fraction. After washing and purification, the sediment was resuspended in 0.25 M sucrose buffer to give 1 g liver weight per 1.25 ml sucrose buffer.

#### **Preparation of kidney lysosomal fraction:**

Kidney lysosomal fraction was prepared according to the method of Shibco and Tappel (1965) and Meisner (1981). After the capsule and fat were removed, the kidneys were longitudinally divided, and then the cortex separated from the remainder of the kidney. The renal (cortex 0.4) gm was then homogenized in 3 ml 0.25 M sucrose buffer and centrifuged (2000 rpm, 5 min, at 4°C). The resulting supernatant fraction was centrifuged (9000 rpm, 15 min at 4°C), the obtained supernatant was stored at 4°C. The lysosomal pellets of every gram of kidney used were then resuspended in 2 ml of 0.25 M sucrose buffer pH 7.4 (Ngha and Ogunleye, 1983).

The activity of the three lysosomal enzymes acid phosphatase (ACP), N-acetyl- $\beta$ -glucosaminidase ( $\beta$ -NAG) and  $\beta$ -galactosidase ( $\beta$ -GAL) has been measured in both liver and kidney according to the method described by Van Hoof and Hers (1968) as modified by Younan and Roseleff (1974).

#### **Statistical Analysis**

The results were expressed as the mean  $\pm$  SE and analyzed for statistical significance by one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons, using SPSS program version 10. Unless otherwise indicated, values were considered statistically significant at  $p < 0.01$  and  $< 0.05$ .

### **3. Results**

#### **Serum markers of liver damage AST, ALT and $\gamma$ -GT activities:**

Table (1) shows that the activities of serum enzymes AST, ALT and  $\gamma$ -GT were markedly increased by the administration of PAR, NIM and LOR in rats either after acute or chronic periods when compared with their control groups. The elevation of liver biomarkers was more pronounced in chronic study than acute one. Moreover, PAR-administration induces liver biomarkers enzymes more than NIM or LOR-administration in both studies.

#### **Serum creatinine and urea levels**

Serum creatinine and urea levels were markedly increased after administration of PAR, NIM or LOR after 4 days (acute) and 4 weeks (chronic) periods when compared with their control groups. Another important observation was that the increases in these parameters were found to be higher in chronic than in acute study (Fig.1).

#### **Hepatic GSH, MDA levels, catalase and SOD activities**

Rats administered with either PAR or NIM resulted in significant reduction of the activities of CAT and SOD and the level of GSH, and significant elevation of the hepatic MDA levels compared to their control groups throughout 4 days and 4 weeks administration (PAR>NIM); and these changes were marked in acute than in chronic study. In contrast, no significant changes were observed in hepatic GSH and MDA levels, Catalase and SOD activities of LOR-administered groups either in acute or chronic periods when compared with their control groups (Fig. 2).

#### **Hepatic lysosomal enzymes (ACP, $\beta$ -NAG and $\beta$ -GAL) activities**

Table (2) reveals that the activities of hepatic lysosomal enzymes ACP,  $\beta$ -NAG and  $\beta$ -GAL were significantly increased in PAR-administered groups followed by NIM-administered groups when compared to either acute or chronic control groups. More pronounced changes in these parameters were obtained in the acute study when compared chronic one. However, no changes in the activities of hepatic lysosomal enzymes were observed in LOR-administered groups in both studies when compared with their control groups.

#### **Renal GSH, MDA levels, catalase and SOD activities**

As indicated in figure (3) PAR, NIM or LOR administration to rats significantly reduced renal GSH level and the activities of the antioxidant enzymes along with the increased levels of renal MDA compared to normal controls in acute and chronic studies. However, LOR-administered group showed no significant alterations in renal in the studied parameters when compared to control groups in acute or chronic studies.

#### **Renal lysosomal enzymes (ACP, $\beta$ -NAG and $\beta$ -GAL) activities**

Renal activities of lysosomal enzymes ACP,  $\beta$ -NAG and  $\beta$ -GAL were significantly increased after the administration of either PAR or NIM to rats when compared to their control groups throughout 4 days and 4 weeks treatment periods. A significant rise in renal activities of enzymes ACP,  $\beta$ -NAG and  $\beta$ -GAL were observed after PAR-administration in comparison with NIM-administration, in addition, PAR or NIM administration to animals was found to induce significant increase in these parameters in acute than in chronic study. However, the activities of renal lysosomal enzymes were not significantly altered after LOR-administration in both studies when compared with their control groups (Table 3).

**Table (1):** Effect of daily administration of paracetamol (PAR) (500mg/kg), nimesulide (NIM) (18mg/kg ) and lornoxicam (LOR) (1.4mg/kg ) for 4 days (acute) and 4 weeks (chronic) on AST, ALT and  $\gamma$ -GT activities in (U/L) in rat serum in comparison with their normal controls (NC).

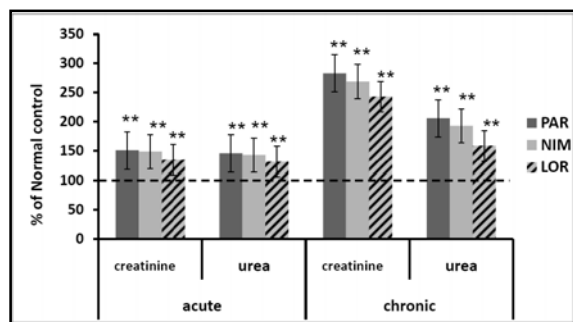
Groups parameter	Acute				Chronic			
	NC	PAR	NIM	LOR	NC	PAR	NIM	LOR
AST	38.34±1.9	64.01**± 2.7	63.89**± 2.7	57.97**± 2.4	38.28± 1.9	95.25**± 2.9	83.69**± 3.1	77.99**± 2.6
ALT	32.33±1.2	56.15**± 2.1	53.09**± 2.6	50.66**± 1.9	35.18± 1.7	79.52**± 2.9	72.04**± 2.4	68.8**± 3.7
$\gamma$ -GT	24.9± 1.3	63.71**± 3.1	58.94**± 2.4	42.43**± 2.5	23.5± 1.7	82.5**± 4.2	81.03**± 3.9	70.5**± 3.6

**Table (2):** Effect of daily administration of paracetamol (PAR) (500mg/kg), nimesulide (NIM) (18mg/kg ) and lornoxicam (LOR) (1.4mg/kg ) for 4 days (acute) and 4 weeks (chronic) on ACP,  $\beta$ -NAG and  $\beta$ -GAL activities in (nmol/ml/h) in rat liver homogenates in comparison with normal controls (NC).

Groups parameter	Acute				Chronic			
	NC	PAR	NIM	LOR	NC	PAR	NIM	LOR
ACP	1243.85±7 3.2	7274.05**± 406.7	5698.63**± 414.8	1321.2± 101.2	1275.2± 57.2	3843.57**± 164.9	3217.28**± 139.8	1296± 49.7
$\beta$ -NAG	415.19± 18.8	1146.59**± 50.1	842.88**± 58.6	543.91± 23.7	452.81± 15.9	840.6**± 56.8	791.23**± 61.01	494.24± 21.5
$\beta$ -GAL	238.31± 10.9	779.32**± 42.5	766.55**± 49.2	250.94± 13.5	283.51± 12.9	742.57**± 32.2	711.62**± 31.4	326.87± 14.6

**Table (3):** Effect of daily administration of paracetamol (500mg/kg), nimesulide (18mg/kg ) and lornoxicam (1.4mg/kg ) for 4 days (acute) and 4 weeks (chronic) on ACP,  $\beta$ -NAG and  $\beta$ -GAL activities in (nmol/ml/h) in rat kidney homogenates in comparison with their normal controls (NC).

Groups parameter	Acute				Chronic			
	NC	PAR	NIM	LOR	NC	PAR	NIM	LOR
ACP	1309.91± 91.4	2695.53**± 199.8	2151.2**± 153.7	1522.37± 117.28	1337.14± 76.6	1741.73**± 76.5	1631.37± 75.5	1480.00± 64.8
$\beta$ -NAG	387.89± 20.2	1532.95**± 66.4	934.92**± 40.7	494.07± 33.6	379.71± 16.8	994.23**± 61.6	787.98**± 65.6	449.94± 19.9
$\beta$ -GAL	317.94± 13.8	705.13**± 30.9	615.6**± 26.2	336.72± 19.9	306.11± 12.8	476.878**± 37.8	454.38**± 23.9	322.62± 13.1

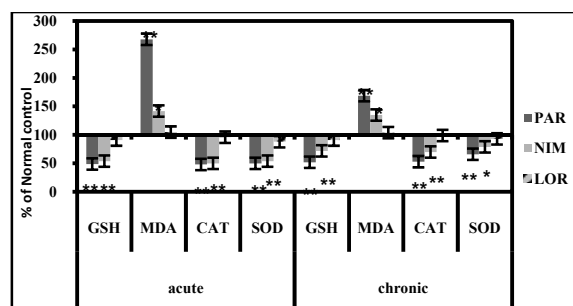


**Fig. (1).** Changes in serum creatinine and urea levels in rats treated with either paracetamol (PAR), nimesulide (NIM) or lornoxicam (LOR) for 4 days (acute) and 4 weeks (chronic).

The values presented as percentage of normal control  $\pm$ SEM of eight rats in each group.

(\*) significantly different from normal control at  $p < 0.05$ .

(\*\*) significantly different from normal control at  $p < 0.01$ .

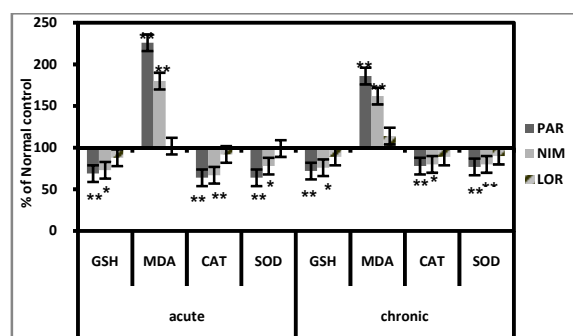


**Fig. (2).** Changes in hepatic content of reduced glutathione (GSH), malondialdehyde (MDA) and the activities of catalase (CAT), superoxide dismutase (SOD) in rats treated with either paracetamol (PAR), nimesulide (NIM) or lornoxicam (LOR) for 4 days (acute) and 4 weeks (chronic).

The values presented as percentage of normal control  $\pm$ SEM of eight rats in each group.

(\*) significantly different from normal control at  $p < 0.05$ .

(\*\*) significantly different from normal control at  $p < 0.01$ .



**Fig. (3).** Changes in renal content of reduced glutathione (GSH), malondialdehyde (MDA) and the activities of catalase (CAT), superoxide dismutase (SOD) in rats treated with either paracetamol (PAR), nimesulide (NIM) or lornoxicam (LOR) for 4 days (acute) and 4 weeks (chronic).

The values presented as percentage of normal control  $\pm$ SEM of eight rats in each group.

(\*) significantly different from normal control at  $p < 0.05$ .

(\*\*) significantly different from normal control at  $p < 0.01$ .

#### 4. Discussion

Hepatotoxicity is a well known reason for drug withdrawal, or delay in the development of safe therapeutics. A number of researches elucidated that NSAIDs are highly liver specific, causing irreversible or reversible toxicity depending on the severity, in humans and animal models (Wallace et al., 2010). The present study showed that the activities of serum enzymes AST, ALT and  $\gamma$ -GT were markedly increased by the administration of PAR, NIM and LOR in rats either after acute or chronic periods when compared with their control groups. The elevation of liver biomarkers was more pronounced in chronic study than acute one. Moreover, PAR-administration induces liver biomarkers enzymes more than NIM or LOR-administration in both studies.

The safety of the chronic use of PAR at therapeutic dose has generated a lot of hot debate (Watkins et al., 2006). At therapeutic doses, PAR is metabolized via glucuronidation and sulfuration reactions occurring primarily in the liver, and results in water-soluble metabolites that are excreted via the kidney. As a result of the metabolic conversion of PAR by the microsomal CYP-450 enzyme system, a highly reactive intermediate, N-acetyl-p-benzoquinoneimine (NAPQI) is produced (Dahlin et al., 1984). NAPQI directly reacts with glutathione (GSH) and at overdoses of PAR, the depletion of cellular GSH occurs which is coincide with our results. This allows NAPQI to bind to cellular proteins and initiate lipid peroxidation, leading to hepatic injury (Dahlin et al., 1984; Kanbur et al., 2009 and Wilhelm et al., 2009) and renal <sup>(31)</sup> injury, which subsequently alters the liver function tests (LFTs) (Nirmala et al., 2012; Sabir et al., 2012 and Salminen et al., 2012).

The current study showed that NIM induced elevated levels of serum activities of AST, ALT and  $\gamma$ -GT associated with adverse reactions in the liver, including hepatocellular necrosis, and/or intrahepatic cholestasis (Boelsterli, 2002a). Licata et al. (2010) found that 70% of the patients had liver damage attributable to NIM. Moreover, the number of patients with elevated aminotransferases during treatment with NIM is, however, increasing, and cases of fulminant and subacute hepatitis, sometimes fatal, have been documented. The formation of nitroso- or hydroxylamine reactive metabolites of NIM has been suggested to be responsible for the liver damage from the drug (Boelsterli, 2002b), like that of reactive metabolite injury from diclofenac, paracetamol and other hepatotoxins (Boelsterli, 2002a). However, there is no evidence to support this reactive metabolite hypothesis of cell injury by NIM. Ong et al. (2006) data confirm and extend earlier studies demonstrating a clear mitochondrial hazard

precipitated by NIM (Moreno-Sanchez et al., 1999; Mingatto et al., 2000 & 2002; and Moreno et al., 2007). Reduction in mitochondrial ATP and other functions has been observed with NIM following administration of high doses of the drug to rats. This phenomenon is related to uncoupling of oxidative phosphorylation like that observed with acidic NSAIDs and might account for the development of liver injury by these drugs. Reduction in ATP may initiate apoptosis by these drugs (Rainsford, 2006 and Tripathi et al., 2010 & 2011).

Our results of elevation of serum levels of aminotransferases by NIM are in accordance with Borku et al. (2008) a case report in a male kitten and many clinical studies of Betrosian et al. (2009).

The current study showed that the increase in serum transaminases levels (AST, ALT and  $\gamma$ -GT) with treatment by LOR may be explained due to LOR has been approved to be eliminated predominantly by hepatic biotransformation. Its major metabolite is the pharmacologically inactive 5V-hydroxylornoxicam (Bonnabry et al., 1996), which accounted for up to 95% of total intrinsic lornoxicam clearance and cytochrome P450 2C9 (CYP2C9) has been proved to be the principal CYP isozyme involved in the main metabolic pathway (Balfour et al., 1996 and Radhofer-Welte and Rabasseda, 2000). This might account for alteration of liver function tests by treatment with LOR.

In the present study, we assessed the nephrotoxic effects caused by acute and chronic administration of different NSAIDs. Our results showed that serum creatinine and urea levels were markedly increased after administration of PAR, NIM or LOR after acute and chronic periods when compared with their control groups, and the rise in these parameters were found to be higher in chronic than in acute study (Fig.2).

Plasma urea and creatinine levels were significantly increased in groups treated with PAR, demonstrating the deterioration of the renal function, in comparison with those of the control group. These findings are consistent with the results of a previous study in which PAR was administered to rats (Fouad et al., 2009 and Ghosh et al., 2010). The mechanisms involved in PAR-induced cell death in nephrotoxicity may differ from hepatotoxicity, as suggested by the fact that N-acetylcysteine can prevent in vivo PAR-induced hepatic damage (Flanagan and Meredith, 1991 and Kozer and Koren, 2001) but did not prevent renal cell death. PAR drug-induced nephrotoxicities are often associated with marked elevations in blood urea nitrogen, serum creatinine and acute tubular necrosis (Verpooten et al., 1998). These findings could be attributed to the ability of PAR overdose to induce an inflammatory reactions with increased

production of  $\alpha$ -tumor necrosis factor (TNF- $\alpha$ ) in the renal tissue (Fouad et al., 2009 and Ghosh et al., 2010). This is in agreement with a number of previous studies which showed that other nephrotoxic drugs can induce renal inflammation with increased generation of (TNF- $\alpha$ ) (Kuhad et al., 2007 and Zager, 2007). Moreover, PAR-induced nephrotoxicity may be explained due to this metabolic activation of PAR to the reactive metabolite, NAPQI (Hart et al., 1994).

Nimesulide treated groups showed significantly elevated serum levels of urea and creatinine which were higher in chronic than in acute study, in the same time they were lower than in PAR groups in both studies when compared with their control groups. Our results are consistent with Borku et al. (2008), who reported a case of nimesulide-induced reversible acute biliary injury and renal failure in a three month old kitten. The results revealed high serum levels of urea and creatinine. Renal adverse effects to this drug may be attributed to the inhibition of prostaglandin synthesis, an increase in renal vascular resistance with a concomitant decrease in diuresis, glomerular filtration rate (GFR), and renal blood flow acute reversible renal failure (Apostolou et al., 1997; Balasubramaniam, 2000 and Prevot et al., 2004).

The results of the current study revealed a remarkable increase in serum levels of urea and creatinine of LOR- treated groups in both studies (acute and chronic), which were found higher in chronic than in acute study, but still lower than PAR and NIM-treated groups when compared with their control groups. LOR had high therapeutic potency and less gastrointestinal side effect when compared to naproxen (Radhofer-Welte and Rabasseda, 2000). Pohlmeier-Esch et al. (1997) investigated different doses of LOR (such as 0.06, 0.16 or 0.40 mg/kg/day) for chronic toxicity in rats. In their study, drug related and dose dependent toxicity of lornoxicam mainly included mortality, reduced body weight gain, some clinicopathological changes (such as anaemia resulting from blood loss), renal damage (renal papillary necrosis) and gastrointestinal mucosal lesions but none of these changes was present after the recovery period. Our study differs from Pohlmeier-Esch et al. (1997) study by administration of higher doses of LOR by intraperitoneal route but not orally. We preferred the dose of 1.4 mg/kg for LOR since it was found to be fully effective to prevent hyperalgesia in rats (Bianchi and Panerai, 2002). According to Sen et al. (2006) the use of LOR alone caused deleterious effects on gastric and renal systems on the fifteenth day which were indicated by histological lesions, where as the administration of lornoxicam and nitroglycerin together prevented these side effects.

The present results indicated in figures 3&5 cleared that PAR-treatment to rats produces a significant elevation of hepatic and renal MDA levels in both studies which is more obvious in acute than chronic study. These findings are consistent with a previous study (Wu et al., 2010). Previous studies have shown that the elevation of hepatic and renal MDA levels can be attributed in part to NAPQI which is the reactive metabolite product caused by PAR-induced hepatotoxicity and nephrotoxicity, leads to GSH depletion and covalently binds to cysteine residues on proteins, which results in lipid peroxidation reaction (Dahlin et al., 1984). Moreover, the increase in the levels of MDA in the PAR-treated group is in agreement with the findings of other researchers (Olaleye and Rocha, 2008; Sabir and Rocha, 2008; Bhadauria and Kumar, 2009; Kanbur et al., 2009; Wilhelm et al., 2009; Fouad and Jresat, 2012 and Sabir et al., 2012). Manimaran et al. (2010) stated that PAR reduced hepatic GSH by 60–90%, indicating inefficient detoxification of NAPQI and its eventual availability for interaction with the cellular macromolecules. Treatment with PAR caused a statistically significant decrease in hepatic and renal SOD activities. Reduction in the activity of SOD is likely to be a result of futile cycling of P450, caused by NAPQI which utilized reducing equivalent of NADPH with concomitant reduction of molecular superoxide anion radical ( $O_2^{\cdot-}$ ), hence there will be a reduction in superoxide dismutase activity (Bessemers and Vermeulen, 2001 and Phil et al., 2012). Moreover, the present results agree with Fouad et al. (2009) who also indicate that increased production of TNF- $\alpha$ , the pro-inflammatory cytokine, in renal tissue that was observed in their study indicates that inflammation is also involved in the pathogenesis of PAR-induced nephrotoxicity and also agree with Cekmen et al. (2009), Ghosh et al. (2010) and Ahmad et al. (2012).

Catalase is a crucial enzyme in cellular antioxidative defense mechanisms and efficiently degrades endogenously produced hydrogen peroxide. Catalase activity was found to be significantly decreased after a toxic PAR dose. Our results are in accordance with Mirochnitchenko et al. (1999), and Rajesh and Parames (2006) who reported that catalase activities were significantly diminished following toxic PAR dose (Fouad and Jresat 2012 and Sabir et al., 2012). This would allow for the accumulation of reactive oxygen species and hydrogen peroxide, which can exacerbate the hepatocellular damage initiated by NAPQI. Also increased reactive oxygen species (ROS) production, impairs mitochondrial respiration, causes ATP depletion, opens the mitochondrial permeability transition pore and makes the mitochondrial inner membrane abruptly

permeable to solutes up to 1500 Da (James et al., 2003 and Jaeschke and Bajt, 2006). These events lead to onset of the mitochondrial permeability transition (MPT), which is a common pathway leading to both necrotic and apoptotic cell death (Yang and Salminen, 2011).

The present results of repeated dosing of PAR-treatment to rats decreases their sensitivity to its hepatotoxic effects, which are associated with oxidative stress and glutathione depletion are in accordance with P J O'Brien (2000).

Resistance to PAR hepatotoxicity and nephrotoxicity produced by repeated exposure to PAR is partially attributable to upregulation of hepatic glucose-6-dehydrogenase (G6PD) and glutathione reductase (GR) activity as an adaptive and protective response to oxidative stress and glutathione depletion. Ghanem et al. (2009) indicates that chronic ingestion of the drug (several tablets per day, for weeks) leads to development of tolerance to the toxic effects of PAR, usually delaying the onset of liver injury. Tolerance to PAR toxicity also occurs in experimental animals such as mice and rats and the mechanism is likely multifactorial (Shayiq et al., 1999; Dalhoff et al., 2001 and Aleksunes et al., 2008). As we mentioned before that PAR is metabolized in the liver mainly by glucuronidation and sulfation, thus generating the non toxic metabolites PAR-glucuronide (PAR-glu) and PAR-sulfate (Thomas, 1993). PAR-glu excretion in bile is mediated by the canalicular multidrug resistance-associated protein 2 (Mrp2) (Xiong et al., 2000). Basolateral efflux of PAR-glu in liver may also occur and has been linked to the expression of Mrp3 (Manautou et al., 2004), an ATP-dependent transporter expressed on the basolateral domain of the hepatocyte (Crocenzi et al., 2004). Ghanem et al. (2005 & 2009) demonstrated that pretreatment with PAR led to a marked increase in the hepatic expression and activity of Mrp3 that was correlated with significant shift from canalicular to basolateral efflux of PAR-glu and a decrease in its enterohepatic recirculation. Thus, increased expression of the basolateral Mrp3 transporter relative to the canalicular Mrp2 transporter is associated with a shift from biliary to basolateral and thereby urinary excretion of PAR-glu. This decrease in enterohepatic recirculation is postulated to contribute to PAR-decreased hepatotoxicity and nephrotoxicity by minimizing exposure to the liver and kidney and PAR activation to the toxic reactive metabolite.

These alterations may contribute to explain the resistance to liver toxicity of the drug, in addition to other well characterized mechanisms (Shayiq et al., 1999; Dalhoff et al., 2001 and Aleksunes et al., 2008).

Rats treated with NIM showed a significant increase in hepatic and renal MDA levels in both studies which is obvious in acute more than chronic study. However, the hepatic and renal MDA levels still lower than PAR-treated groups. Also, our experimental result here showed significant decrease in hepatic and renal GSH level, SOD and CAT activities in acute study which increase gradually in chronic treatment but still significant lower than PAR-treated group when compared with their control groups. This finding is in agreement with Tripathi et al. (2010) (in vitro study) who stated that mitochondria are known to be a major source of intracellular ROS generation and are particularly vulnerable to oxidative stress. We suggest that oxidative damage synchronized with mitochondrial damage and subsequent apoptosis is the main cause of NIM-induced cytotoxicity in hepatic cells. Tripathi et al. (2011) indicated that NIM exposure caused significant alterations in the antioxidant level leading to oxidative stress. Roig et al. (2002) showed that NIM (5 mg/kg/ day) for 8 days reduced renal blood flow in dogs. Nimesulide injected acutely was shown to reduce renal blood flow in newborn rabbits (Prevot et al., 2004). In Al Suleimani et al. (2010) study, a combination of nimesulide and cisplatin (CP) exaggerated renal tissue damage, as demonstrated by histopathology in rats.

LOR-treated groups showed insignificant changes in hepatic MDA levels, GSH content as well as hepatic SOD and CAT activities in both studies which indicate that LOR may cause liver damage without oxidative stress. These findings are in accordance with Ayan et al. (2008) which demonstrated that LOR can inhibit oxidative tissue damage in the trachea and the lungs induced with direct intratracheal application of peroxynitrite and with Sen et al. (2006) who also reported that CAT and GSH levels increased in LOR-treated group compared to those in control groups. It was demonstrated that oxicams are more reactive against ROS than NIM and ibuprofen in rats (Van Antwerpen, 2004) and moreover LOR appears to be a significantly better antioxidant than tenoxicam. It was stated that the antioxidant properties of lornoxicam might be related to its chemical structure. Bulbuloglu et al. (2005) concluded that the use of lornoxicam was effective in decreasing the oxidative stress of tissue during peritonitis. Based on these data, it is assumed that although LOR may cause liver and kidney damage but without ROS formation. According to Baliga et al. (2010) study, it was shown that LOR in a dose of 16 mg/day had an early onset of action and a better tolerability profile as compared to diclofenac 150 mg/day in the treatment of adult Indian patients with osteoarthritis. It could be

therefore a safer and alternative option in the symptomatic treatment of patients with osteoarthritis with lesser dosing frequency. They believe that this effect of LOR is not only a result of cyclo-oxygenase inhibition but also is attributable to its nitric oxide (NO) and superoxide inhibitory properties as mentioned in previous studies (Pruss et al., 1990 and Radhofer-Welte and Rabasseda, 2000).

Our results in figures 4&6 concerning lysosomal enzymes are consistent with Khandkar et al. (1996) which suggest that PAR toxicity specifically induced the synthesis of the lysosomal enzymes and moreover, Bhadauria and Kumar (2009) suggested that PAR toxicity specifically induced the synthesis of the lysosomal enzymes in both organs (liver and kidney). The present results also showed NIM-treated groups produce significant increase in hepatic and renal lysosomal enzymes activities (ACP,  $\beta$ -NAG and  $\beta$ -GAL) in both studies but in acute more than chronic, on the other hand, NIM-treated groups showed lesser significant lysosomal enzymes activities than PAR-treated groups when compared with their normal controls. As we discussed above that lysosomal enzymes may increase under pathological conditions and under the effects of some xenobiotics (e.g. drugs) which leads to labialization of the lysosomal membrane which leads to leakage of lysosomal enzymes (Khandkar et al., 1996 and Sabir and Rocha, 2008).

However, LOR-treated groups in this study showed insignificant changes in hepatic lysosomal enzymes activities (ACP,  $\beta$ -NAG and  $\beta$ -GAL) in both studies when compared with their control groups. The reason for this normalcy may be attributed to the anti-inflammatory effect of the drug, probably due to stabilization of the lysosomal membrane (Poonguzhali et al., 1998).

Poonguzhali et al. (1998) showed that tenoxicam, which is a member of oxicams family produce stabilization of lysosomal membrane which may be attributed to the anti-inflammatory effect of the drug, probably. These findings indicate that LOR may exert the same effects as tenoxicam on liver and kidney lysosomal membranes. Also as shown above in the present results, LOR-treated groups showed insignificant changes in the antioxidant system which indicate that LOR does not produce ROS that may alters the stability of lysosomal membranes as well as the cell membranes. These alterations in lysosomal enzymes may contribute to explain the hepatotoxicity and nephrotoxicity under the effects of some NSAIDS drugs which leads to labialization of the lysosomal membrane which leads to leakage of lysosomal enzymes (Khandkar et al., 1996 and Bhadauria and Kumar, 2009).

Lornoxicam treated groups in this study showed insignificant changes in hepatic lysosomal enzymes activities (ACP,  $\beta$ -NAG and  $\beta$ -GAL) in both studies when compared with their control groups.

The results concerning repeated exposure to PAR and NIM were consistent with those of Ghanem et al. (2005 & 2009) studies which indicate that the alterations in the antioxidant system in both studies (acute and chronic) may contribute to explain the resistance to liver and kidney toxicity of both drugs (PAR and NIM).

Excessive lysosomal enzymes synthesis may be attributing to the membrane lipids damage. In the present study, a rise was found in the liver and renal lysosomal enzymes activity which appears to be a cause of increased membrane lysis and cell damage. This effect is exaggerated with changes in the antioxidant system in liver and kidney.

### Conclusions

In conclusions, the present study showed that paracetamol is the most hepatotoxic and nephrotoxic drug than nimesulide and lornoxicam. Resistance to xenobiotic hepatotoxicity and nephrotoxicity produced by repeated exposure (chronic) is partially attributable to upregulation of antioxidant defense system activity as an adaptive and protective response to oxidative stress. Also, these results indicate that lornoxicam may induce hepatotoxicity and nephrotoxicity but not associated with oxidative stress. Among the treated groups studied, only lornoxicam showed that the least changes in most of the parameters measured in the current study. These findings suggest that LOR may hold potential as promising approaches for treatment of human with the least side effects than other NSAIDs.

### Abbreviations:

ACP: acid phosphatase  
 ANOVA: Analysis of variance  
 ALT: Alanine aminotransferase  
 AST: Aspartate aminotransferase  
 ATP: Adenosine triphosphate  
 CAT: catalase  
 G6PD: Glucose-6-dehydrogenase  
 GFR: Glomerular filtration rate  
 GR: Glutathione reductase  
 GSH: Reduced Glutathione (L- $\gamma$ -glutamyl-L-cysteinyl glycine)  
 LFTs: liver function test  
 LOR: lornoxicam  
 MDA: Malondialdehyde  
 MPT: Mitochondrial permeability transition  
 Mrp: Multidrug resistance associated protein  
 NAPQI: N-acetyl-p-benzoquinoneimine  
 NO: nitric oxide  
 NSAIDs: Non-steroidal anti-inflammatory drugs  
 PAR: paracetamol

PMN: Polymorphnuclear  
 ROS: reactive oxygen species  
 rpm: rotation per minute  
 SE: standard error  
 SOD: superoxide dismutase  
 TNF- $\alpha$ : Tumor necrosis factor- $\alpha$   
 $\beta$ -GAL:  $\beta$ -Galactosidase  
 $\beta$ -NAG: N-Acetyl  $\beta$ -D-Glucosaminidase  
 $\gamma$ -GT: Gamma-glutamyltransferase

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## Synthesis and docking studies of furobenzopyrones of potential antimicrobial and photochemotherapeutic activities

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**Abstract:** Benzopyrone and furobenzopyrone derivatives were designed to be synthesized and screened for antimicrobial and photosensitizing activities. Synthesis of benzopyrone derivatives (**IVa-e** and **Va-d**) was proceeded via etherification of hydroxy benzopyrone **I** and **II** with  $\omega$ -bromoacetophenone derivatives **IIIa-e** followed by cyclization to achieve linear furobenzopyrone analogues (**IVa-e** and **Va-d**). Surprisingly, an angular furobenzopyrone derivative **VIII** instead of the linear analogue was synthesized in one step reaction from the condensation of hydroxy benzopyrone **II** with 3,4-dimethoxy- $\omega$ -bromoacetophenone **IIIe**. This may be attributed to the presence of the two methoxy substituents which are electron donating group. All newly synthesized compounds were evaluated for their antimicrobial and photosensitizing activities by the paper disc diffusion method compared with xanthotoxin. Results showed that, compounds **IVd**, **IVe**, **Vd**, **VIIa** and **VIII** possessed antimicrobial and potential photosensitizing activity. Compounds **IVe** and **VIII** exhibited antimicrobial activity higher than that of xanthotoxin while the other three compounds were less active than xanthotoxin. Docking of the antimicrobial active compounds into topoisomerase II using MOE program was performed in order to predict the correlation between dock scores and antimicrobial activity of these compounds.

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### 1. Introduction

Photochemotherapy is used for treatment of hyperproliferative skin diseases, (psoriasis, mycosis fungoides or cavitary tumors). In skin diseases, a combination of psoralen **1** (**Figure 1**) and radiation in the interval of UV-A (320-400 nm), is called PUVA [therapy derived from psoralens (linear furocoumarins) and UV-A] [1].

Furocoumarin and their derivatives are well known photosensitizing drugs for the treatment of some skin diseases (e.g., psoriasis, vitiligo, mycosis and eczema) [2-4], as well as bacterial, fungal [5] and viral infections [6-8]. Moreover, it was reported that psoralen derivatives had applications in the treatment of cutaneous T-cell lymphoma [9], human immunodeficiency disease [10,11] and prevention of organ transplants rejection [12].

Psoralen tricyclic moiety constitutes the basic chromophore of drugs employed in this therapy, in particular, 8-methoxy psoralen [8-MOP] **2**, 5-Methoxy psoralen [5-MOP] **3** and to a lesser extent the synthetic 4,5',8-trimethylpsoralen [TMP] **4**, **Figure 1** [1].

Photosensitizing drugs [13] used in photochemotherapy were proved to act through various mechanisms. Type **I** implies substrate photo-oxidation by radical species. Both activated oxygen species (superoxide anion and hydroxyl radical) and

radical species are formed by electron transfer photo excited furocoumarins. Type **II** involves generation of singlet oxygen by energy transfer process [14]. In the type **III** mechanism, the triplet photosensitizer may react directly with a substrate in an oxygen independent process [15]. The main biological and therapeutic effects of psoralens are generally attributed to type **III** mechanism, and in particular to photoreaction with DNA due to presence of olefinic double bonds [16,17].

This photo reactive process seems to take place in three phases: i Insertion between adjacent pyrimidine base pairs in the DNA duplex (occurs in the dark). ii Absorption of one photon by psoralen induces the formation of monoadducts with the neighboring pyrimidine via interaction of the respective carbon-carbon double bonds that both compounds have (two types of monoadduct, pyrone type and furan type). iii Monoadduct absorb another photon, inducing its other photo reactive double bond to interact with a thymidine on the opposite strand of DNA, therefore a diadduct, that cross linked the DNA helix, is formed resulting in interstrand cross linkage. Cross linkage provokes more pronounced biological consequences, but repair of interstrand cross linkage is less effective than repair of the monofunctional adduct [18-20].

Linear furobenzopyrones are reported to induce bifunctional photodamage to the DNA of the cutaneous cells in a selective way, thus inhibiting DNA functions and as a consequence, the cell proliferation. The photodamages consist of the products of photocycloaddition between one molecule of psoralen and two pyrimidine bases of (biadduct). Therefore, 2,3 (furan side) and 5,6 (pyrone) double bonds of the furobenzopyrones are the two photoreactive sites responsible for the DNA photobinding and for the biological activity [20].

The therapeutic treatment, however, is accompanied by some undesirable side effects such as skin phototoxicity and risk of skin cancer. Skin phototoxicity is strictly connected with the bifunctional lesions in DNA which seems to be the main cause of the risk of skin cancer. On the other hand, monoadducts are reported to lack skin phototoxicity.

It is reported that, genotoxicity is the undesirable side effect of linear furobenzopyrones and it is developed from the formation of DNA bifunctional adduct [21]. Therefore DNA monofunctional furobenzopyrone such as 6-carbomethoxy **5** [22], pyrido **6** [22], benzo **7** [23] analogues (**Figure 1**) have been designed and synthesized in order to prevent DNA interstrand cross link formation, consequently lack skin phototoxicity and at the same time maintain the photosensitizing activity. In a previous work, a phenyl substituent at C5 in furobenzopyrones increased the photosensitizing activity [24].

Examination of the model of intercalation complex between furobenzopyrones and nucleic acid revealed that the C5 methyl of thymidine and the C5 substitution of the furobenzopyrone are in close proximity. Thus, the presence of a methyl group in this position could lead to steric crowding not present in the demethyl case [25]. The results reported about TMP **4** and psoralen **1** lend further support to this interpretation [TMP showed  $\approx 98\%$  furan furan addition, while psoralen lacking a methyl at 5-position, showed nearly  $\approx 20\%$  pyrone addition].

The previous information enforced us to design and synthesize new linear furobenzopyrone substituted at position 5 and 6 with methyl groups to act as monofunctional agents thus decrease the side effect (phototoxicity) of these novel analogues.

## 2. Experimental

### 2.1. General remarks

Melting points were determined by open capillary tube method using Electrothermal 9100 melting point apparatus MFB-595-010M (Gallen Kamp, London, England) and were uncorrected. Microanalysis was carried out at The Regional Center for Mycology and Biotechnology, Al-Azhar

University. Infrared Spectra were recorded as potassium bromide discs on Shimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan). The  $^1\text{H}$  NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer at 300 MHz and \*JEOL-ECA500 NMR spectrometer at 500 MHz in dimethylsulphoxide ( $\text{DMSO}-d_6$ ) or  $\text{CDCl}_3$ . Chemical Shifts are quoted in  $\delta$  as parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard and  $J$  values are reported in Hz. Mass spectra were performed as EI at 70eV on Hewlett Packard Varian (Varian, Palo, USA) and Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX. TLC were carried out using Art.DC-Plastikfolien, Kieselgel 60 F254 sheets (Merck, Darmstadt, Germany), the developing solvents was chloroform/methanol 9.5:0.5 and the spots were visualized at 366, 254 nm by UV Vilber Lourmat 77202 (Vilber, Marne La Vallee, France).

### 2.2. Chemistry

Starting compounds 3,4-Dimethyl-7-hydroxy-8-substituted-2*H*-benzopyran-2-one **I**, **II** [26-28] and  $\omega$ -bromoacetophenone derivatives **IIIa-e** [29] were prepared according to reported procedures.

#### 2.2.1. General Procedure for synthesis of 3,4-dimethyl-8-substituted-7-(3,4-disubstituted phenacyloxy)-2*H*-benzopyran-2-one (**IVa-e** and **Va-d**):

A solution of **I** or **II** (0.01 mol) and  $\omega$ -bromoacetophenone derivatives **IIIa-e** (0.015 mol) in acetone (50 mL) was refluxed in presence of anhydrous potassium carbonate (2.76 g, 0.02 mol) for 24 h. Acetone was distilled then the solid products were filtered, washed and dried to yield 57-95%.

##### 2.2.1.1. 7-Phenacyloxy-3,4,8-trimethyl-2*H*-benzopyran-2-one (**IVa**):

Yield 84%. The crude product was crystallized from isopropanol. Mp 195 – 196 °C. IR  $\nu_{\text{max}}$ /  $\text{cm}^{-1}$ : 3100 (CH Ar), 2964, 2872 (CH aliphatic), 1710, 1697 (2 C=O), 1606, 1568, 1556, 1508 (C=C).  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  ppm: 2.08 (s, 3H,  $\text{CH}_3$ ), 2.27 (s, 3H,  $\text{CH}_3$ ), 2.35 (s, 3H,  $\text{CH}_3$ ), 5.77 (s, 2H,  $\text{CH}_2$ ), 6.98 (d, 1H,  $J=8.7$  Hz, H-6 Ar), 7.48-7.70 (m, 4H, H-3',4',5' Ar and H-5 Ar), 8.02 (d, 2H,  $J=7.2$  Hz, H-2',6' Ar). MS ( $m/z$ ) %: 322 ( $\text{M}^+$ ) 15.03%. Anal. Calcd. For  $\text{C}_{20}\text{H}_{18}\text{O}_4$  (322.35): C, 74.52; H, 5.63. Found: C, 74.59; H, 5.61.

##### 2.2.1.2. 7-(4-Methylphenacyloxy)-3,4,8-trimethyl-2*H*-benzopyran-2-one (**IVb**):

Yield 91%. The crude product was crystallized from isopropanol. Mp 249 – 250 °C. IR  $\nu_{\text{max}}$ /  $\text{cm}^{-1}$ : 3059 (CH Ar), 2922, 2858 (CH aliphatic), 1710, 1683 (2 C=O), 1602, 1573, 1500 (C=C).  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  ppm: 2.07 (s, 3H,  $\text{CH}_3$ ), 2.27 (s, 3H,  $\text{CH}_3$ ), 2.34 (s, 3H,  $\text{CH}_3$ ), 2.39 (s, 3H,  $\text{CH}_3$ ), 5.71 (s, 2H,  $\text{CH}_2$ ), 6.95 (d, 1H,  $J=8.7$  Hz, H-6 Ar), 7.38 (d, 2H,  $J=7.8$  Hz, H-3',5' Ar), 7.54 (d, 1H,  $J=8.7$  Hz, H-5 Ar), 7.92 (d, 2H,  $J=8.1$  Hz, H-2',6' Ar). MS ( $m/z$ ) %: 336 ( $\text{M}^+$ ) 8.99 %, Anal. Calcd. For

$C_{21}H_{20}O_4$  (336.38): C, 74.98; H, 5.99. Found: C, 75.03; H, 6.06.

**2.2.1.3. 7-(4-Methoxyphenacyloxy)-3,4,8-trimethyl-2H-benzopyran-2-one (IVc):** Yield 75%. The crude product was crystallized from isopropanol. Mp 169 – 170 °C. IR  $\nu_{max}/cm^{-1}$ : 3070 (CH Ar), 2922, 2843 (CH aliphatic), 1708, 1693 (2 C=O), 1602, 1575, 1520, 1500 (C=C).  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 2.07 (s, 3H, CH<sub>3</sub>), 2.26 (s, 3H, CH<sub>3</sub>), 2.34 (s, 3H, CH<sub>3</sub>), 3.85 (s, 3H, OCH<sub>3</sub>), 5.68 (s, 2H, CH<sub>2</sub>), 6.94 (d, 1H,  $J=8.7$  Hz, H-6 Ar), 7.09 (d, 2H,  $J=9$  Hz, H-3',5' Ar), 7.54 (d, 1H,  $J=8.7$  Hz, H-5 Ar), 8.00 (d, 2H,  $J=8.7$  Hz, H-2',6' Ar). MS ( $m/z$ ) %: 352 ( $M^+$ ) 12.28 %. Anal. Calcd. For  $C_{21}H_{20}O_5$  (352.38): C, 71.58; H, 5.72. Found: C, 71.58; H, 5.76.

**2.2.1.4. 7-(4-Bromophenacyloxy)-3,4,8-trimethyl-2H-benzopyran-2-one (IVd):** Yield 80%. The crude product was crystallized from isopropanol. Mp 240 – 242 °C. IR  $\nu_{max}/cm^{-1}$ : 3060 (CH Ar), 2964, 2872 (CH aliphatic), 1710, 1695 (2 C=O), 1606, 1579, 1554 (C=C).  $^1H$  NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 2.19 (s, 3H, CH<sub>3</sub>), 2.31 (s, 3H, CH<sub>3</sub>), 2.36 (s, 3H, CH<sub>3</sub>), 5.31 (s, 2H, CH<sub>2</sub>), 6.70 (d, 1H,  $J=8.7$  Hz, H-6 Ar), 6.88 (d, 1H,  $J=8.7$  Hz, H-5 Ar), 7.67 (d, 2H,  $J=8.7$  Hz, H-3',5' Ar), 7.88 (d, 2H,  $J=8.7$  Hz, H-2',6' Ar). MS ( $m/z$ ) %: 401 ( $M^+$ ) 5.95%, 403 ( $M^++2$ ) 5.52%. Anal. Calcd. for  $C_{20}H_{17}BrO_4$  (401.25): C, 59.87; H, 4.27. Found: C, 59.92; H, 6.04.

**2.2.1.5. 7-(3,4-Dimethoxyphenacyloxy)-3,4,8-trimethyl-2H-benzopyran-2-one (IVe):** Yield 71%. The crude product was crystallized from isopropanol. Mp 208 – 209 °C. IR  $\nu_{max}/cm^{-1}$ : 3060 (CH Ar), 2960, 2860 (CH aliphatic), 1710, 1697 (2 C=O), 1602, 1571 (C=C).  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 2.07 (s, 3H, CH<sub>3</sub>), 2.27 (s, 3H, CH<sub>3</sub>), 2.34 (s, 3H, CH<sub>3</sub>), 3.82 (s, 3H, OCH<sub>3</sub>), 3.86 (s, 3H, OCH<sub>3</sub>), 5.70 (s, 2H, CH<sub>2</sub>), 6.93 (d, 1H,  $J=9.3$  Hz, H-6 Ar), 7.11 (d, 1H,  $J=9$  Hz, H-5' Ar), 7.48 (s, 1H, H-2' Ar), 7.54 (d, 2H,  $J=9$  Hz, H-5 Ar), 7.72 (d, 1H,  $J=8.4$  Hz, H-6' Ar). MS ( $m/z$ ) %: 382 ( $M^+$ ) 36.11%. Anal. Calcd. For  $C_{22}H_{22}O_6$  (382.41): C, 69.10; H, 5.80. Found: C, 69.08; H, 5.83.

**2.2.1.6. 3,4-Dimethyl-7-phenacyloxy-2H-benzopyran-2-one (Va):** Yield 85%. The crude product was crystallized from isopropanol. Mp 178 – 179 °C. IR  $\nu_{max}/cm^{-1}$ : 3057 (CH Ar), 2912, 2860 (CH aliphatic), 1703, 1693 (2 C=O), 1624, 1614, 1581, 1566 (C=C).  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 2.07 (s, 3H, CH<sub>3</sub>), 2.37 (s, 3H, CH<sub>3</sub>), 5.73 (s, 2H, CH<sub>2</sub>), 7.01 (d, 1H,  $J=9$  Hz, H-6 Ar), 7.05 (s, 1H, H-8 Ar), 7.58 (t, 3H, H-3',4',5' Ar), 7.70 (d, 1H,  $J=9$  Hz, H-5 Ar), 8.04 (d, 2H,  $J=10.2$  Hz, H-2',6' Ar). MS ( $m/z$ ) %: 308 ( $M^+$ ) 18.14%. Anal. Calcd. For  $C_{19}H_{16}O_4$  (308.33): C, 74.01; H, 5.23. Found: C, 74.13; H, 5.28.

**2.2.1.7. 3,4-Dimethyl-7-(4-methylphenacyloxy)-2H-benzopyran-2-one (Vb):** Yield 95%. The crude product was crystallized from isopropanol. Mp 173 –

176 °C. IR  $\nu_{max}/cm^{-1}$ : 3007 (CH Ar), 2958, 2872 (CH aliphatic), 1708, 1695 (2 C=O), 1602, 1562, 1508 (C=C).  $^1H$  NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 2.18 (s, 3H, CH<sub>3</sub>), 2.36 (s, 3H, CH<sub>3</sub>), 2.45 (s, 3H, CH<sub>3</sub>), 5.34 (s, 2H, CH<sub>2</sub>), 6.78 (s, 1H, H-8 Ar), 6.94 (d, 1H,  $J=8.7$  Hz, H-6 Ar), 7.32 (d, 1H,  $J=8.4$  Hz, H-3',5' Ar), 7.51 (d, 1H,  $J=9$  Hz, H-5 Ar), 7.89 (d, 2H,  $J=8.4$  Hz, H-2',6' Ar). MS ( $m/z$ ) %: 322 ( $M^+$ ) 13.21%. Anal. Calcd. For  $C_{20}H_{18}O_4$  (322.35): C, 74.52; H, 5.63. Found: C, 74.49; H, 5.65.

**2.2.1.8. 3,4-Dimethyl-7-(4-methoxyphenacyloxy)-2H-benzopyran-2-one (Vc):** Yield 57%. The crude product was crystallized from isopropanol. Mp 167 – 170 °C. IR  $\nu_{max}/cm^{-1}$ : 3072 (CH Ar), 2960, 2839 (CH aliphatic), 1710, 1689 (2 C=O), 1604, 1577, 1566, 1508 (C=C).  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 2.06 (s, 3H, CH<sub>3</sub>), 2.36 (s, 3H, CH<sub>3</sub>), 3.86 (s, 3H, OCH<sub>3</sub>), 5.64 (s, 2H, CH<sub>2</sub>), 6.99 (d, 1H,  $J=8.7$  Hz, H-6 Ar), 7.01 (s, 1H, H-8 Ar), 7.09 (d, 1H,  $J=9$  Hz, H-3',5' Ar), 7.69 (d, 1H,  $J=8.4$  Hz, H-5 Ar), 8.01 (d, 2H,  $J=8.7$  Hz, H-2',6' Ar). MS ( $m/z$ ) %: 338 ( $M^+$ ) 1.07%. Anal. Calcd. For  $C_{20}H_{18}O_5$  (338.35): C, 70.99; H, 5.36. Found: C, 71.08; H, 5.38.

**2.2.1.9. 7-(4-Bromophenacyloxy)-3,4-dimethyl-2H-benzopyran-2-one (Vd):** Yield 85%. The crude product was crystallized from isopropanol. Mp 219 – 220 °C. IR  $\nu_{max}/cm^{-1}$ : 3064 (CH Ar), 2920, 2866 (CH aliphatic), 1710, 1697 (2 C=O), 1610, 1583, 1568, 1535 (C=C).  $^1H$  NMR (CDCl<sub>3</sub>)  $\delta$  ppm: 2.19 (s, 3H, CH<sub>3</sub>), 2.37 (s, 3H, CH<sub>3</sub>), 5.30 (s, 2H, CH<sub>2</sub>), 6.80 (s, 1H, H-8 Ar), 6.93 (d, 1H,  $J=8.7$  Hz, H-6 Ar), 7.52 (d, 1H,  $J=8.7$  Hz, H-5 Ar), 7.67 (d, 2H,  $J=7.8$  Hz, H-3',5' Ar), 7.87 (d, 1H,  $J=7.8$  Hz, H-2',6' Ar). MS ( $m/z$ ) %: 387 ( $M^+$ ) 0.15%. Anal. Calcd. for  $C_{19}H_{15}BrO_4$  (387.22): C, 58.93; H, 3.90. Found: C, 59.02; H, 3.94.

**2.2.2. General Procedure for synthesis of 5,6-dimethyl-9-substituted-3-(3,4-disubstituted phenyl)-7H-furo[3,2-g]benzopyran-7-one (VIa-e and VIIa-d):** Compound IVa-e or Va-d (0.01 mol) was added to solution of 2% potassium hydroxide in absolute ethanol (50 mL) and the mixture was refluxed for 18 h. Solution was concentrated and acidified with a cold solution of 10 % HCl. The precipitated product was filtered, washed and dried to yield 55-87%.

**2.2.2.1. 3-Phenyl-5,6,9-trimethyl-7H-furo[3,2-g]benzopyran-7-one (VIa):** Yield 87%. The crude product was crystallized from isopropanol. Mp 213 – 215 °C. IR  $\nu_{max}/cm^{-1}$ : 3026 (CH Ar), 2920, 2825 (CH aliphatic), 1701 (C=O), 1629, 1593, 1566 (C=C).  $^1H$  NMR (DMSO- $d_6$ )  $\delta$  ppm: 2.11 (s, 3H, CH<sub>3</sub>), 2.45 (s, 3H, CH<sub>3</sub>), 2.49 (s, 3H, CH<sub>3</sub>), 7.42 (t, 1H, H-4' Ar), 7.53 (t, 2H, H-3',5' Ar), 7.76 (d, 2H,  $J=7.8$  Hz, H-2',6' Ar), 7.90 (s, 1H, H-4 Ar), 8.39 (s, 1H, H-2 Ar). MS

(*m/z*) %: 304 ( $M^+$ ) 100%. Anal. Calcd. For  $C_{20}H_{16}O_3$  (304.34): C, 78.93; H, 5.30. Found: C, 79.15; H, 5.36.

**2.2.2.2. 3-(4-Methylphenyl)-5,6,9-trimethyl-7H-furo[3,2-g]benzopyran-7-one (VIb):** Yield 83%. The crude product was crystallized from isopropanol. Mp 222 – 225 °C. IR  $\nu_{\max}/\text{cm}^{-1}$ : 3113 (CH Ar), 2966, 2873 (CH aliphatic), 1708 (C=O), 1612, 1591, 1566, 1510 (C=C).  $^1\text{H NMR}^*$  ( $\text{CDCl}_3$ )  $\delta$  ppm: 2.24 (s, 3H,  $\text{CH}_3$ ), 2.43 (s, 3H,  $\text{CH}_3$ ), 2.45 (s, 3H,  $\text{CH}_3$ ), 2.62 (s, 3H,  $\text{CH}_3$ ), 7.32 (d, 2H,  $J=7.65$  Hz, H-3',5' Ar), 7.52 (d, 2H,  $J=8.4$  Hz, H-2',6' Ar), 7.78 (s, 1H, H-4 Ar), 7.80 (s, 1H, H-2 Ar). MS (*m/z*) %: 318 ( $M^+$ ) 0.4%. Anal. Calcd. For  $C_{21}H_{18}O_3$  (318.13): C, 79.22; H, 5.70. Found: C, 79.28; H, 5.79.

**2.2.2.3. 3-(4-Methoxyphenyl)-5,6,9-trimethyl-7H-furo[3,2-g]benzopyran-7-one (VIc):** Yield 58%. The crude product was crystallized from isopropanol. Mp 236 – 238 °C. IR  $\nu_{\max}/\text{cm}^{-1}$ : 3007 (CH Ar), 2926, 2829 (CH aliphatic), 1710 (C=O), 1616, 1591, 1570, 1508 (C=C).  $^1\text{H NMR}^*$  ( $\text{CDCl}_3$ )  $\delta$  ppm: 2.23 (s, 3H,  $\text{CH}_3$ ), 2.45 (s, 3H,  $\text{CH}_3$ ), 2.64 (s, 3H,  $\text{CH}_3$ ), 3.87 (s, 3H,  $\text{OCH}_3$ ), 7.05 (d, 2H,  $J=8.4$  Hz, H-3',5' Ar), 7.55 (d, 2H,  $J=8.45$  Hz, H-2',6' Ar), 7.74 (s, 1H, H-4 Ar), 7.76 (s, 1H, H-2 Ar). MS (*m/z*) %: 334 ( $M^+$ ) 1.19%. Anal. Calcd. For  $C_{21}H_{18}O_4$  (334.37): C, 75.43; H, 5.43. Found: C, 75.48; H, 5.50.

**2.2.2.4. 3-(4-Bromophenyl)-5,6,9-trimethyl-7H-furo[3,2-g]benzopyran-7-one (VIId):** Yield 72%. The crude product was crystallized from isopropanol. Mp 265 – 268 °C. IR  $\nu_{\max}/\text{cm}^{-1}$ : 3107 (CH Ar), 2928, 2838 (CH aliphatic), 1705 (C=O), 1590 (C=C).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm: 2.26 (s, 3H,  $\text{CH}_3$ ), 2.47 (s, 3H,  $\text{CH}_3$ ), 2.63 (s, 3H,  $\text{CH}_3$ ), 7.51 (d, 2H,  $J=8.4$  Hz, H-2',6' Ar), 7.65 (d, 2H,  $J=8.4$  Hz, H-3',5' Ar), 7.76 (s, 1H, H-4 Ar), 7.82 (s, 1H, H-2 Ar). MS (*m/z*) %: 383 ( $M^+$ ) 0.58%. Anal. Calcd. For  $C_{20}H_{15}\text{BrO}_3$  (383.24): C, 62.68; H, 3.95. Found: C, 62.71; H, 3.93.

**2.2.2.5. 3-(3,4-Dimethoxyphenyl)-5,6,9-trimethyl-7H-furo[3,2-g]benzopyran-7-one (VIe):** Yield 63%. The crude product was crystallized from isopropanol. Mp 203 – 206 °C. IR  $\nu_{\max}/\text{cm}^{-1}$ : 3092 (CH Ar), 2942, 2843 (CH aliphatic), 1697 (C=O), 1620, 1593, 1560 (C=C).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm: 2.26 (s, 3H,  $\text{CH}_3$ ), 2.47 (s, 3H,  $\text{CH}_3$ ), 2.65 (s, 3H,  $\text{CH}_3$ ), 3.97 (s, 6H,  $2 \times \text{OCH}_3$ ), 7.03 (d, 1H,  $J=8.1$  Hz, H-5' Ar), 7.13 (s, 1H, H-2' Ar), 7.21 (d, 1H,  $J=9$  Hz, H-6' Ar), 7.78 (s, 1H, H-4 Ar), 7.81 (s, 1H, H-2 Ar). MS (*m/z*) %: 364 ( $M^+$ ) 2.05%. Anal. Calcd. For  $C_{22}H_{20}O_5$  (364.39): C, 72.51; H, 5.53. Found: C, 72.49; H, 5.58.

**2.2.2.6. 5,6-Dimethyl-3-phenyl-7H-furo[3,2-g]benzopyran-7-one (VIIa):** Yield 77%. The crude product was crystallized from isopropanol. Mp 215 – 216 °C. IR  $\nu_{\max}/\text{cm}^{-1}$ : 3082 (CH Ar), 2929, 2860 (CH aliphatic), 1714 (C=O), 1627, 1606, 1577 (C=C).  $^1\text{H NMR}^*$  ( $\text{CDCl}_3$ )  $\delta$  ppm: 2.24 (s, 3H,  $\text{CH}_3$ ), 2.47 (s, 3H,  $\text{CH}_3$ ), 7.43 (t, 1H, H-4' Ar), 7.46 (s, 1H, H-9 Ar), 7.52

(t, 2H, H-3',5' Ar), 7.63 (d, 1H,  $J=6.9$  Hz, H-2',6' Ar), 7.80 (s, 1H, H-4 Ar), 7.90 (s, 1H, H-2 Ar). MS (*m/z*) %: 290 ( $M^+$ ) 98.64%. Anal. Calcd. For  $C_{19}H_{14}O_3$  (290.31): C, 78.61; H, 4.86. Found: C, 78.68; H, 4.91.

**2.2.2.7. 5,6-Dimethyl-3-(4-methylphenyl)-7H-furo[3,2-g]benzopyran-7-one (VIIb):** Yield 76%. The crude product was crystallized from isopropanol. Mp 251 – 253 °C. IR  $\nu_{\max}/\text{cm}^{-1}$ : 3080 (CH Ar), 2960, 2860 (CH aliphatic), 1690 (C=O), 1640, 1580, 1550 (C=C).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm: 2.26 (s, 3H,  $\text{CH}_3$ ), 2.45 (s, 3H,  $\text{CH}_3$ ), 2.49 (s, 3H,  $\text{CH}_3$ ), 7.35 (d, 1H,  $J=8.4$  Hz, H-3',5' Ar), 7.49 (s, 1H, H-9 Ar), 7.54 (d, 1H,  $J=7.8$  Hz, H-2',6' Ar), 7.79 (s, 1H, H-4 Ar), 7.97 (s, 1H, H-2 Ar). MS (*m/z*) %: 304 ( $M^+$ ) 100%. Anal. Calcd. For  $C_{20}H_{16}O_3$  (304.34): C, 78.93; H, 5.30. Found: C, 79.04; H, 5.31.

**2.2.2.8. 5,6-Dimethyl-3-(4-methoxyphenyl)-7H-furo[3,2-g]benzopyran-7-one (VIIc):** Yield 55%. The crude product was crystallized from isopropanol. Mp 197 – 198 °C. IR  $\nu_{\max}/\text{cm}^{-1}$ : 3060 (CH Ar), 2931, 2839 (CH aliphatic), 1697 (C=O), 1598, 1570, 1508 (C=C).  $^1\text{H NMR}^*$  ( $\text{CDCl}_3$ )  $\delta$  ppm: 2.17 (s, 3H,  $\text{CH}_3$ ), 2.41 (s, 3H,  $\text{CH}_3$ ), 3.89 (s, 3H,  $\text{OCH}_3$ ), 6.77 (s, 1H, H-9 Ar), 6.92 (d, 2H,  $J=8.4$  Hz, H-3',5' Ar), 7.50 (d, 2H,  $J=9.1$  Hz, H-2',6' Ar), 7.96 (s, 1H, H-4 Ar), 7.98 (s, 1H, H-2 Ar). MS (*m/z*) %: 320 ( $M^+$ ) 0.95%. Anal. Calcd. For  $C_{20}H_{16}O_4$  (320.34): C, 74.99; H, 5.03. Found: C, 75.03; H, 5.08.

**2.2.2.9. 3-(4-Bromophenyl)-5,6-dimethyl-7H-furo[3,2-g]benzopyran-7-one (VIId):** Yield 83%. The crude product was crystallized from isopropanol. Mp 242 – 245 °C. IR  $\nu_{\max}/\text{cm}^{-1}$ : 3074 (CH Ar), 2924, 2860 (CH aliphatic), 1708 (C=O), 1608, 1585, 1571, 1508 (C=C).  $^1\text{H NMR}$  ( $\text{CDCl}_3$ )  $\delta$  ppm: 2.21 (s, 3H,  $\text{CH}_3$ ), 2.39 (s, 3H,  $\text{CH}_3$ ), 6.99 (s, 1H, H-9 Ar), 7.41 (s, 1H, H-4 Ar), 7.54 (d, 2H,  $J=8.4$  Hz, H-2',6' Ar), 7.62 (d, 2H,  $J=8.1$  Hz, H-3',5' Ar), 7.87 (s, 1H, H-2 Ar). MS (*m/z*) %: 369 ( $M^+$ ) 3.92%. Anal. Calcd. For  $C_{19}H_{13}\text{BrO}_3$  (369.21): C, 61.81; H, 3.55. Found: C, 61.90; H, 3.49.

**2.2.3. Synthesis of 3-(3,4-dimethoxyphenyl)-6,7-dimethyl-5H-furo[2,3-h]benzopyran-5-ones (VIII):** Previous procedure adopted for synthesis of 3,4-dimethyl-8-substituted-7-(3,4-disubstituted phenacyloxy)-2H-benzopyran-2-one (IVa-e and Va-d) was applied on reacting 3,4-dimethyl-7-hydroxy-2H-benzopyran-2-one **II** and 3,4-dimethoxy- $\omega$ -bromoacetophenone **IIIe** except that reaction was proceeded for 18 h instead of 24 h.

Yield 59%. The crude product was crystallized from isopropanol. Mp 169 – 171 °C. IR  $\nu_{\max}/\text{cm}^{-1}$ : 3080 (CH Ar), 2922, 2852 (CH aliphatic), 1691 (C=O), 1612, 1550 (C=C).  $^1\text{H NMR}$  ( $\text{DMSO}-d_6$ )  $\delta$  ppm: 2.07 (s, 3H,  $\text{CH}_3$ ), 2.36 (s, 3H,  $\text{CH}_3$ ), 3.79 (s, 3H,  $\text{OCH}_3$ ), 3.86 (s, 3H,  $\text{OCH}_3$ ), 7.08-7.17 (m, 3H, H-2',5',6' Ar), 7.55 (s, 1H, H-2 Ar), 7.76 (d, 1H,  $J=8.7$  Hz, H-9 Ar),

7.84 (d, 1H,  $J=8.4$  Hz, H-8 Ar). MS ( $m/z$ ) %: 350 ( $M^+$ ) 16.72%. Anal. Calcd. For  $C_{21}H_{18}O_5$  (350.36): C, 71.99; H, 5.18. Found: C, 71.96; H, 5.22.

### 2.3. Antimicrobial and photosensitizing activity:

Nine benzopyrone derivatives and ten furobenzopyrone analogues (nine linear and one angular) were screened for antimicrobial and photosensitizing activity by the paper disc diffusion method [30] compared with xanthotoxin, as a reference compound, that has been clinically investigated. The tested organism used was *Bacillus subtilis*.

In the preliminary test, employing strong condition (high concentration of the substance) was used for selecting the active compounds, even if weakly active, from the inactive ones.

In another test, only active compounds were tested to determine the effect of concentration and the time of radiation (exposure to UV-A) on their photosensitizing activity, which was compared with xanthotoxin as a reference compound.

#### Pre-experimental preparations:

- Nutrient agar medium: 0.3% of the beef extract, 0.5% of peptone, 0.1% of dipotassium hydrogen phosphate and 1.5% agar.
- Broth culture of the organism: slant agar seeded with the tested organism (*Bacillus subtilis*), incubated overnight and the broth culture of the organism was prepared.
- Paper disc: Whatman no. 1 filter paper disc (5mm) were sterilized and impregnated with the different concentrations of the tested compounds which were dissolved in dimethylformamide (DMF), and allowed to dry overnight. Two concentrations were prepared from each of the tested compounds.

#### Experimental

0.02 mL of the prepared broth culture was added carefully in the sterile petri dishes then 10 mL of the liquefied nutrient agar medium were added, allowed to be mixed uniformly and solidified. The impregnated discs were arranged uniformly on the solidified agar layer. Each plate contained disc impregnated with DMF (neglect effect of the solvent) and another disc impregnated with xanthotoxin as reference compound.

Two groups of plates were used, one as test plates was incubated in the dark at 37°C for 3 h before irradiation to allow for diffusion of the tested compounds through the agar layer, and the duplicate plates were left in the incubator overnight as control to determine the antimicrobial activity.

Covers were removed from the plates of the first group (tested petri dishes) and the dishes were exposed to U.V. lamp (365 nm) for 20 min. After irradiation, the plates were reincubated in the dark at 37 °C overnight and examined for antimicrobial and

photosensitizing activity by measuring the produced inhibition zones. Results are presented in **Table 1, Figure 2**.

The experiment was repeated using the selected active compounds to study the effect of concentration and time of radiation on the photosensitizing activity.

Two groups of discs were prepared. One group of the discs was impregnated with 0.01 mL (each disc contained 0.5 mg of the tested compounds) and the other group was impregnated with 0.02 mL (each disc contained 1 g of the tested compounds). Results are presented in **Table 2, Figure 3**.

### 2.4. Molecular docking

#### Docking procedure

Docking studies of all the synthesized compounds were performed by molecular operating Environment (MOE) 2008.10 release of Chemical Computing Group, Canada. [31] The program operated under "Window XP" operating system installed on an Intel Pentium IV PC with a 2.8 MHz processor and 512 RAM. All minimizations were performed with MOE until a RMSD gradient of 0.05 Kcal mol<sup>-1</sup> Å<sup>-1</sup> with MMFF94 force field and the partial charges were automatically calculated. The score function, dock function (S, Kcal/mol) developed by MOE program was used for the evaluation of the binding affinity of the ligand.

#### Preparation of the target topoisomerase II

The X-ray crystal structure of the enzyme with benzopyrone ligand (PDB code 1AJ6) [32] was obtained from the protein data bank in PDB format. The enzyme was prepared for docking studies.

- 3D protonation for the amino acid side chain and Novobiocin.
- Deleting all water of crystallization away from the active site.
- Isolation of the active site, fixation to be dealt with as rigid structure and recognition of the amino acids.
- Creation of dummies around the active site.
- Studying the interactions of the ligand (Novobiocin) with the amino acids of the active site.

#### Preparation of compounds for docking:

The 3D structures of the synthesized compounds were built using MOE and subjected to the following procedure: (i) 3D protonation of the structures. (ii) Running conformational analysis using systemic search. (iii) Selecting the least energetic conformer. (iv) Applying the same docking protocol used with Novobiocin.

#### Docking running

Prior to the docking of the benzopyrone derivatives, redocking of the native ligand bound in the topoisomerase II active site was performed to validate the docking protocol. The generated most stable conformer of each compound was virtually docked into the predefined active site of

topoisomerase II. The developed docked models were energetically minimized and then used to predict the interaction of the ligand with the amino acids in the active site of the enzyme.

### 3. Results and Discussion

#### 3.1. Chemistry

The targeted compounds **VIa-e**, **VIIa-d**, and **VIII** were synthesized as illustrated in **Scheme 1**. The intermediate compounds 3,4-dimethyl-8-substituted-7-(3,4-disubstituted phenacyloxy)-2*H*-benzopyran-2-one **IVa-e** and **Va-d** were prepared following procedure adopted by Musajo *et al.* [33] to avoid any probability for the opening of the sensitive pyrone ring. Reaction was processed via refluxing 7-hydroxy-2*H*-benzopyran-2-one **I** or **II** with  $\omega$ -bromoacetophenone derivatives **IIIa-e** in dry acetone containing anhydrous potassium carbonate. The new ether derivatives gave negative ferric chloride test. The proposed structures were confirmed by spectral and analytical data. The IR spectrum revealed absence of band corresponding to phenolic OH at 3300  $\text{cm}^{-1}$  and presence of two bands at 1710-1703 and 1697-1683  $\text{cm}^{-1}$  corresponding to C=O of pyrone ring and ketone C=O, respectively.  $^1\text{H}$  NMR revealed presence of a singlet signal at  $\delta = 5.30\text{-}5.77$  ppm assigned to  $\text{CH}_2$  group confirming ether formation in addition to increased number of aromatic protons. MS spectra showed appearance of their molecular ion peaks.

Cyclization of ether derivatives **IVa-e** and **Va-d** to the corresponding furo[3,2-g]benzopyran-7-ones **VIa-e** and **VIIa-d** were achieved through reflux with alcoholic potassium hydroxide followed by subsequent acidification. The structures of the synthesized compounds were confirmed by spectral and analytical data. IR spectrum showed only one band at 1714-1690  $\text{cm}^{-1}$  corresponding to C=O of pyrone ring.  $^1\text{H}$  NMR revealed disappearance of singlet peak assigned to  $\text{CH}_2$ , two doublet peaks of the H-6 and H-5 aromatic protons, respectively and appearance of two singlet peak corresponding to H-4 and H-2 at 7.41-7.96 and 7.76-8.39 ppm, respectively. Presence of these two singlet peaks instead of two doublets and one singlet peaks (in case of derivatives with unsubstituted 8- position) confirmed formation of linear furobenzopyranone. MS spectra showed appearance of their molecular ion peaks.

Attempts for etherification of 3,4-dimethyl-7-hydroxy-2*H*-benzopyran-2-one **II** with 3,4-dimethoxy- $\omega$ -bromoacetophenone **IIIe** under previous conditions gave the angular 3-(3,4-dimethoxyphenyl)-6,7-dimethyl-5*H*-furo[2,3-h]benzopyran-5-one **VIII** in one step reaction. Trials to decrease reaction time to obtain the ether derivative gave same product. The structure was deduced by spectral and analytical data. IR showed absence of band corresponding to phenolic OH present in starting compound.  $^1\text{H}$  NMR revealed

presence of two singlets at 3.79 and 3.86, one singlet at 7.55 and two doublets at 7.76 and 7.84 assigned to 3',4' methoxy groups, H-2, H-9 and H-8, respectively. In addition, absence of singlet signal assigned to  $\text{CH}_2$  protons confirmed etherification followed by subsequent cyclization and formation of angular furobenzopyranone. MS spectra showed presence of molecular ion peak at 350.

#### 3.2. Antimicrobial and photosensitizing activity:

The result of preliminary experiment showed that, linear furobenzopyrone derivative **VIIa** and benzopyrone derivatives **IVe** and **Vd** possessed antimicrobial and potential photosensitizing activity while compounds such as benzopyrone derivative **IVd** and angular furobenzopyrone **VIII** had antimicrobial activity only. Moreover, compounds **IVe** and **VIII** (both had dimethoxy substitution) exhibited antimicrobial activity higher than that of xanthotoxin while the other three compounds were less active than xanthotoxin. The rest of the prepared new compounds were inactive.

The study of time and concentration effect on photosensitizing activity revealed that, increase in time of exposure to light increased photosensitizing activity in all tested compounds except for compound **VIII** while increase in concentration increased photosensitizing activity in all tested compounds. Benzopyrone derivatives (containing bromo substituent) **IVd** and **Vd** and linear furobenzopyrone derivative **VIIa** (contained unsubstituted phenyl at C3) possessed photosensitizing activity greater than xanthotoxin.

#### 3.3. Molecular docking

Topoisomerases are enzymes that control the changes in DNA structure by catalyzing the breaking and rejoining of the phosphodiester backbone of DNA strands during the normal cell cycle. Topoisomerases became targets for cancer chemotherapy treatments as topoisomerase inhibitors block the ligation step of the cell cycle, generating single and double stranded breaks in DNA and subsequently lead to apoptosis and cell death. Topoisomerase inhibitors can also function as antibacterial agents [34]. Topoisomerase inhibitors are often divided according to which type of enzyme they inhibit to Topoisomerase I inhibitors and Topoisomerase II inhibitors. There are two subclasses of type II topoisomerases, type IIA and IIB. Type IIA topoisomerases form double-stranded breaks with four-base pair overhangs and able to simplify DNA topology, while type IIB topoisomerases form double-stranded breaks with two base overhangs and do not simplify DNA topology [35]. Small molecules that target type II topoisomerase are divided into two classes: inhibitors and poisons. Inhibitors of type II topoisomerase as mitindomide work by inhibiting the ATPase activity by acting as a non-competitive



inhibitor of ATP. Poisons of type II topoisomerases such as Novobiocin target the DNA-protein complex and lead to increased cleavage, whereas others, such as etoposide, inhibit religation. Topoisomerase poisons are used as both anticancer and antibacterial therapies [36].

The binding affinity of the ligand was evaluated with energy score (S, Kcal/mol). The compound which revealed the highest binding affinity, minimum dock score, is the one forming the most stable ligand-enzyme complex. Length of the hydrogen bond and arene cation interaction were also used to assess the binding models. The results of docking studies: dock score, involved topoisomerase II active site amino acid interacting ligand moieties and hydrogen bond length for each compound and ligand are listed in **Table 3, Figures 4-8**.

Analysis of the docking results revealed that:

i) Novobiocin-topoisomerase II complex was precisely reproduced by the docking procedure as demonstrated by low root mean standard deviation, rmsd (0.6204) and dock score (-13.6636 Kcal/mol, **Table 3**), i.e. the docking protocol was valid. As shown in **Figure 4**, Novobiocin nearly fits in the active site forming various hydrogen bonding interactions with the active site residues: CO carbamate with **Thr 165**, **Gly 77** and **Asp 73** (2.02 Å) through a water molecule, CO benzopyrone with **Gly 77** (1.82 Å) through a water molecule, CO amide with **Arg 76** (2.04 Å) through a water molecule, NH<sub>2</sub> carbamate with **Asp 73** (1.91 Å) and **Val 43** (2.30 Å) through a water molecule and OH pyrane with **Asn 46** (2.05 Å). Also Novobiocin forms arene cation interaction of benzene of benzopyrone with **Arg 76**.

ii) From the dock scores, all compounds were found to have negative dock score ranging from -12.7145 to -11.0298 Kcal/mol. It means that most of compounds formed stable complex with enzyme. A significant correlation between dock scores and antimicrobial activity of the compounds was observed. For benzopyrone derivatives **IVd**, **IVe** and **Vd** (dock score, -11.0737, -12.0769 and -11.0759 Kcal/mol, respectively), linear furobenzopyrone derivative **VIIa** (dock score, -11.0298 Kcal/mol) and angular furobenzopyrone **VIII** (dock score, -12.7145 Kcal/mol), the highest negative dock score among all tested compounds was estimated for the derivatives **VIII** and **IVe** (with dimethoxy substitution) that exhibited higher antimicrobial activity than xanthotoxin. Other tested compounds exhibited antimicrobial activity lower than xanthotoxin. These results were attributed to involvement of 3',4'-dimethoxy groups in hydrogen bonding with amino acids in active site of the enzyme.

iii) Inspection of the binding mode also demonstrated that all compounds showed from one to

six hydrogen bonds and arene cation interaction with the enzyme active site residue. **Thr 165**, **Gly 77**, **Arg 76**, **Asp 73** and **Val 43** are the amino acid residues involved in this interaction and **Gly 77** is the common residue involved in this interaction (**Table 3** and **Figures 5-8**).

Regarding the angular furobenzopyrone **VIII** with lowest energy score (-12.7145 Kcal/mol), the most active compound, mediated four strong hydrogen bonds with **Thr 165** (2.92 Å), **Gly 77** through a water molecule (1.74 Å) and **Asp 73**, through a water molecule (1.74 Å) through 3'-methoxy group and **Thr 165** through a water molecule (2.48 Å), through 4'-methoxy group (**Table 3, Figures 5** and **6**).

Regarding the benzopyrone **IVe** with low energy score (-12.0769 Kcal/mol), the second most active compound mediated six strong hydrogen bonds with **Thr 165** (1.84 Å) through a water molecule, **Gly 77** (1.84 Å) through a water molecule and **Asp 73** (1.84 Å) through a water molecule through 3'-methoxy group, **Val 43** through water molecule (3.61 Å) through 4'-methoxy group and **Arg 76** (1.95 Å) and through a water molecule (2.57 Å) through CO benzopyrone (**Table 3, Figures 7** and **8**).

**Table 1: Preliminary screening of substituted benzopyrone and furobenzopyrone derivatives as antimicrobial and photosensitizing agents.**

Compound	Control*	Test**
DMF	---	---
IVa	---	---
IVb	---	---
IVc	---	---
IVd	6	6
IVe	17	18
Va	---	---
Vb	---	---
Vc	---	---
Vd	8	10
VIa	---	---
VIb	---	---
VIc	---	---
VId	---	---
VIe	---	---
VIIa	6	10
VIIb	---	---
VIIc	---	---
VIIId	---	---
VIII	21	21
Xanthotoxin	9	12

\*Disk contains 0.01 mL of the tested and reference compounds.

\*\*Disk contains 0.01 mL of the tested and reference compounds and time of radiation is 20 min.

**Table 2: Antimicrobial and photosensitizing activity of substituted benzopyrone and furobenzopyrone derivatives.**

Comp. No.	Control*	Test**	Test***	Test****
DMF	---	---	---	---
IVd	6	6	12	18
IVe	17	18	22	25
Vd	8	10	14	19
VIIa	6	10	14	17
VIII	21	21	21	26
Xanthotoxin	9	12	15	19

\*Disk contains 0.01 mL of the tested and reference compounds.

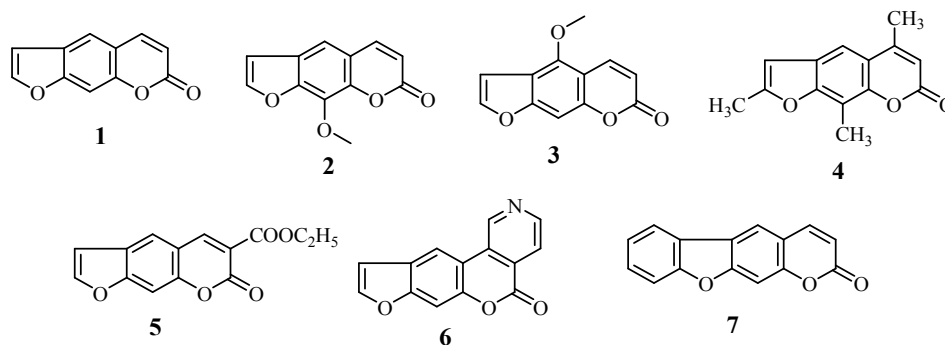
\*\*Disk contains 0.01 mL of the tested and reference compounds and time of radiation is 20 min.

\*\*\*Disk contains 0.01 mL of the tested and reference compounds and time of radiation is 40 min.

\*\*\*\*Disk contains 0.02 mL of the tested and reference compounds and time of radiation is 20 min.

**Table 3: Docking results**

Compound	Energy score S (Kcal/mol)	Binding amino acid	Interacting function group	Hydrogen bond length Å
Novobiocin	-13.6636	Thr 165 (through water molecule)	CO carbamate	2.02
		Gly 77 (through water molecule)	CO carbamate	2.02
		Gly 77 (through water molecule)	CO benzopyrone	1.82
		Arg 76 (cation-arene)	Benzene of benzopyrone	
		Arg 76 (through water molecule)	CO amide	2.04
		Asp 73 (through water molecule)	CO carbamate	2.02
		Asp 73	NH <sub>2</sub> carbamate	1.91
		Asn 46	OH pyrane	2.05
		Val 43 (through water molecule)	NH <sub>2</sub> carbamate	2.30
IVd	-11.0737	Thr 165 (through water molecule)	CO acyloxy	1.82
		Gly 77 (through water molecule)	CO acyloxy	1.82
		Arg 76 (cation-arene)	Benzene of benzopyrone	
		Asp 73 (through water molecule)	CO acyloxy	1.82
IVe	-12.0769	Thr 165 (through water molecule)	3'-OCH <sub>3</sub>	1.84
		Gly 77 (through water molecule)	3'-OCH <sub>3</sub>	1.84
		Arg 76	CO benzopyrone	1.95
		Arg 76 (through water molecule)	CO benzopyrone	2.57
		Asp 73 (through water molecule) Val 43 (through water molecule)	3'-OCH <sub>3</sub>	1.84
			4'-OCH <sub>3</sub>	3.61
Vd	-11.0759	Thr 165	CO benzopyrone	3.44
		Gly 77 (through water molecule)	O acyloxy	2.06
		Gly 77 (through water molecule)	CO acyloxy	1.49
		Arg 76 (cation-arene)	Benzene of benzopyrone	
VIIa	-11.0298	Arg 76 (cation-arene)	Phenyl at C <sub>2</sub>	
		Val 43 (through water molecule)	CO benzopyrone	3.69
VIII	-12.7145	Thr 165	3'-OCH <sub>3</sub>	2.92
		Thr 165 (through water molecule)	4'-OCH <sub>3</sub>	2.48
		Gly 77 (through water molecule)	3'-OCH <sub>3</sub>	1.74
		Asp 73 (through water molecule)	3'-OCH <sub>3</sub>	1.74

**Figure 1: Furobenzopyrone compounds**

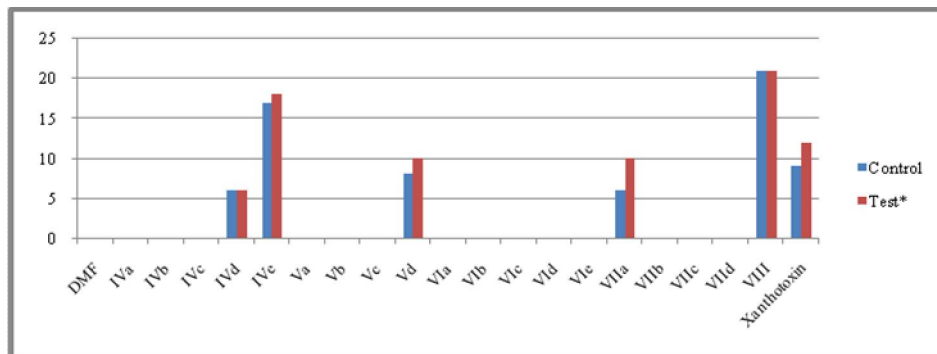


Figure 2: The bar diagram showing antimicrobial and photosensitizing activity of the tested compounds and their comparison to solvent DMF and Xanthotoxin.

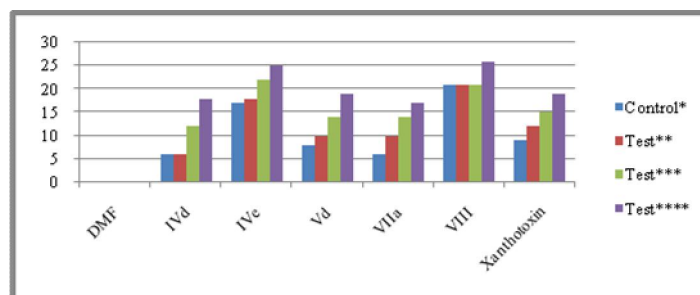


Figure 3: The bar diagram showing effect of increase in time of radiation and concentration of solution on photosensitizing activity of the tested compounds and their comparison to solvent DMF and Xanthotoxin.

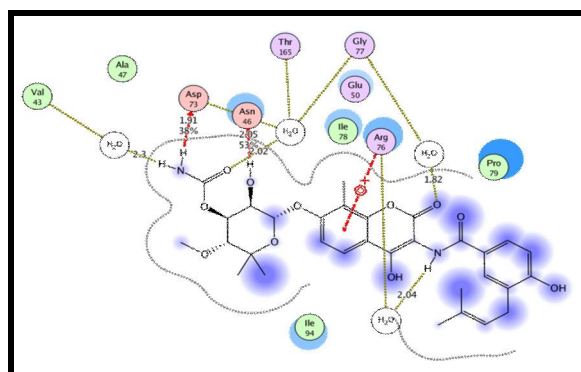


Figure 4: 2D interactions of Novobiocin on the active site of Topoisomerase II

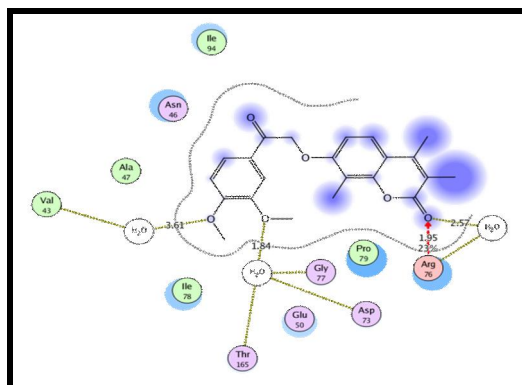


Figure 5: 2D interactions of compound IVe on the active site of Topoisomerase II

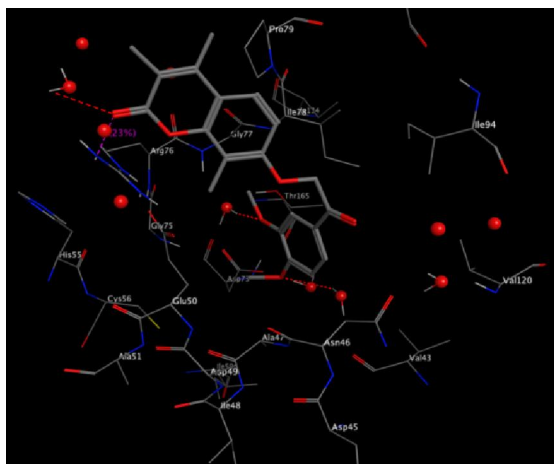


Figure 6: 3D interactions of compound IVe on the active site of Topoisomerase II

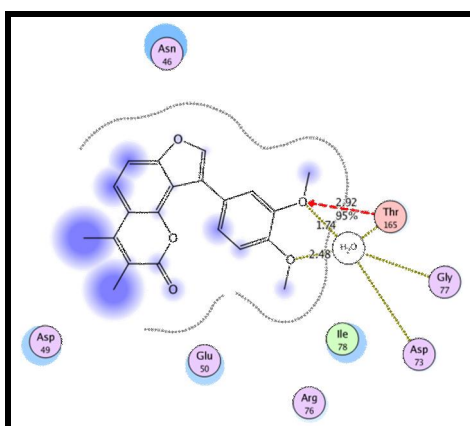


Figure 7: 2D interactions of compound VIII on the active site of Topoisomerase II

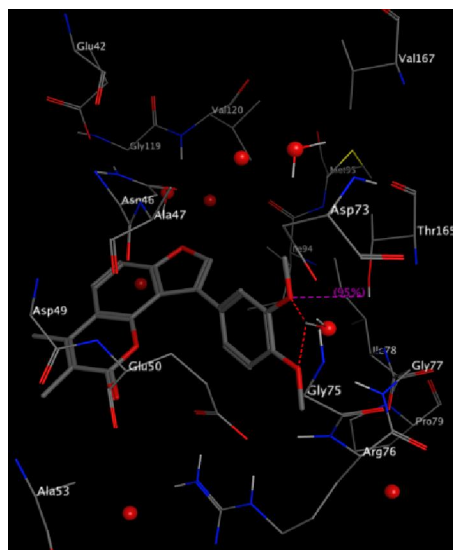
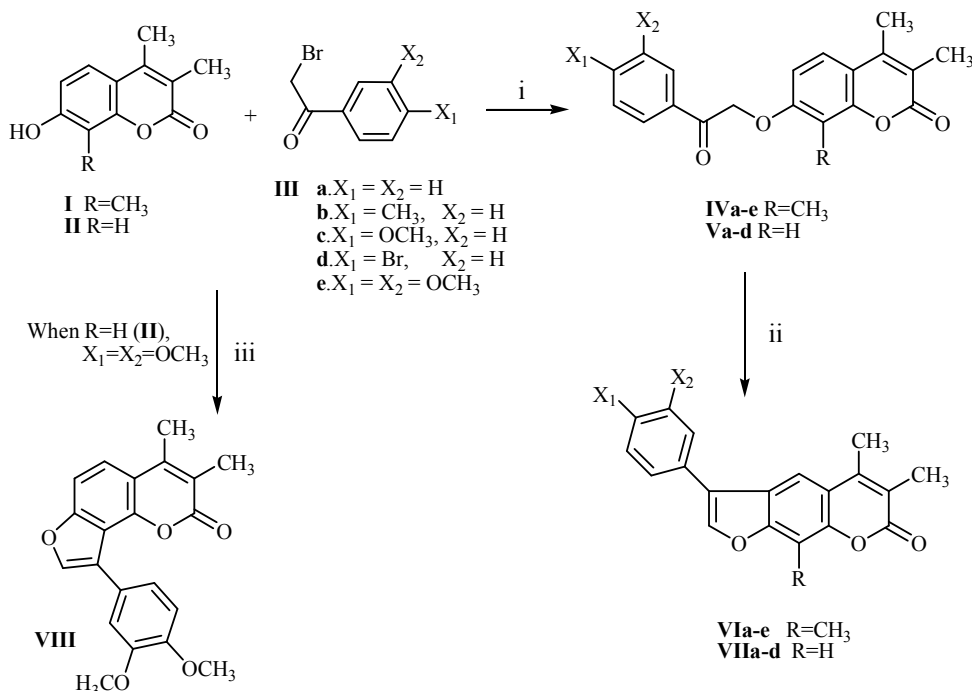


Figure 8: 3D interactions of compound VIII on the active site of Topoisomerase II



**Scheme 1.** Reagents and conditions: (i) K<sub>2</sub>CO<sub>3</sub>, dry acetone, reflux 24 h, (ii) Ethanolic KOH, reflux 18 h, (iii) K<sub>2</sub>CO<sub>3</sub>, dry acetone, reflux 18 h

#### 4. Conclusion

From the previous results of antimicrobial and photosensitizing screening we concluded that:

The two methoxy groups (compounds **IVe** and **VIII** were active and even higher than xanthoxin as antimicrobial) and their orientation towards carbonyl of benzopyrone (**VIe** was inactive) are important factors that enhanced antimicrobial activity.

Benzopyrone derivatives containing bromo substitution needed longer time of exposure to light or high concentration to exhibit photosensitizing activity (compounds **IVd** and **Vd** were active as antimicrobial lower than xanthoxin but exhibited photosensitizing activity higher than xanthotoxin on increasing time of exposure to radiation or increasing concentration of solution used).

Substitution on phenyl ring present at C3 of linear furobenzopyrone derivative abolished activity as only compound **VIIa** (contained unsubstituted phenyl at C3) possessed antimicrobial activity less than xanthotoxin although the photosensitizing activity was good.

Therefore, docking of the antimicrobial active compounds into topoisomerase II using MOE program revealed a correlation between dock scores and experimental antimicrobial activity of these compounds. Presence of two methoxy groups in compounds **IVe** and **VIII** provided hydrogen bonds to amino acids in active site of topoisomerase II enzyme

and this may be the reason for their high antimicrobial activity.

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## Phytochemical and Biological Investigation of Leaf Extracts of *Podocarpus gracilior* and *Ruprechtia polystachya* Resulted in Isolation of Novel Polyphenolic Compound

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**Abstract:** Phytochemical investigation of polyphenolic contents of *Podocarpus gracilior* Pilger and *Ruprechtia polystachya* Griseb leaves were resulted in isolation and identification of three and six known polyphenolic compounds respectively. In addition of a new polyphenolic compound isolated for the first time from nature from *R. polystachya* which is identified as 4'-*O*-Galloyl-myricetin-3-*O*- $\alpha$ -L-rhamnopyranoside (4'-*O*-galloyl myricetrin). Identification of hydrocarbons in *P. gracilior* and *R. polystachya* leaves resulted in identification of 19 and 21 compounds respectively. Concerning the composition of fatty acids content in *P. gracilior* it could be concluded that the unsaturated fatty acids (51.16%) represented higher percentage than that of saturated ones (38.87%). In the case of *R. polystachya*, the saturated fatty acids (70.58%) were dominated on the unsaturated ones (27.30%). The tested methanol extracts of *P. gracilior* and *R. polystachya* leaves showed antioxidant, antimicrobial and stimulatory activities to nitric oxide release from macrophage cell line. Methanol extracts of *P. gracilior* leaves had weak cytotoxic effect against MCF-7 cells (breast adenocarcinoma cell line) while methanol extracts of *R. polystachya* leaves did not show cytotoxic activity.

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**Key words:** *Ruprechtia polystachya*, *Podocarpus gracilior*, polyphenols, antioxidant, cytotoxic, antimicrobial and anti-inflammatory.

### 1. Introduction:

Plants containing, flavonoids and/or tannins received considerable attention for their biological activities<sup>[1-4]</sup>. For example some species of *Podocarpus* genus (family Podocarpaceae) and *Ruprechtia* genus (family Polygonaceae) reported to have several biological activities like antioxidant, cytotoxic, anti-inflammatory, antiviral, antimicrobial activities. These biological activities were revealed for their contents of terpenoid, flavonoids and tannins<sup>[5-10]</sup>. So in this study it was interesting to take an overview of two interesting species growing in Egypt each from one of the previous mentioned families. Like *Podocarpus gracilior* (Family Podocarpaceae) (*Pg*) which was reported as antioxidant and detected to contain Taxol,<sup>[6&11]</sup> this could be used as anticancer. Another plant is *Ruprechtia polystachya* (family: Polygonaceae) (*Rp*) which has an inhibitory activity on the glucose-6-phosphatase system, so may be used as antidiabetic drug<sup>[10]</sup>.

### 2. Material and Methods:

#### Apparatus

JOEL GX-500 (500 and 125 MHz for <sup>1</sup>H and <sup>13</sup>C NMR), NMR department, National Research Center (NRC), and NMR in Strathclyde Institute of Pharmacy and Biomedical Science, Glasgow, United Kingdom were used. The  $\delta$ -values were reported as

ppm relative to TMS in DMSO-*d*<sub>6</sub> and *J*-values were in Hz. ESI-MS spectra were measured on Walters ACQUITY/TQD triple quadrupole, Center for Applied Research and Advanced Studies, Faculty of Pharmacy Cairo University. The UV analyses for pure samples were recorded on a Shimadzu UV 240 spectrophotometer, separately as solutions in methanol and with different diagnostic UV shift reagents<sup>[12&13]</sup>. Rotary evaporator (Büchi, G, Switzerland). Fractionation of the extracts was done by columns chromatography using polyamide 6S (Riedel-De Hën Ag, Seelze Hannover, Germany), isolation and purification of compounds were done on either cellulose (Pharmacia, Uppsala, Sweden) or Sephadex LH-20 (Fluka, Switzerland) columns of different dimensions and eluted with different solvent systems (Figures 1 and 2). Separation processes were followed up by 2D-PC and CoPC using Whatmann No. 1 paper with (S<sub>1</sub>) and (S<sub>2</sub>) as in table 1. Ultraviolet lamp (VL-215 LC, Marne La Vallee, France): It was used for visualization of spots on paper and thin layer chromatograms and follow up the columns fractionation on columns at 254 and/or 365 nm and also with sprayed Naturstoff reagent<sup>[14]</sup>. Gas liquid chromatography TRACE GC ULTRA was used for analysis of both total fatty acids (TFA) and unsaponifiable matter (USM) using GC/MS HP 6890 series (Agilent) MSD, Faculty of Agriculture, Cairo University according to the following conditions:

Capillary column HP6890 series (30 m x 0.25 mm i.d. and 0.25  $\mu$ m film thickness); detector: MSD; carrier gas: Helium, with flow rate: 1 ml/min; injector temperature: 270°C; detector temperature: 280°C; initial column temperature: 70°C, programmed by 8°C/min up to final temperature 270°C within 20 min. GLC conditions for total fatty acids analysis: Capillary column: Thermo TR-FAME (70% cyanopropyl polysilphenylene siloxane) (30 m x 0.25 mm i.d. and 0.25  $\mu$ m film thickness); detector: flame ionization; carrier gas: N<sub>2</sub>, with flow rate 30 ml/min; injector temperature: 200°C; detector temperature: 220°C; initial column temperature: 140°C, programmed by 5°C/min up to final temperature 200°C within 12 min. Fluostar Optima microplate ELISA reader and 96 well cell culture microplates were used for pharmacological studies.

#### Plant material

Leaves of *Ruprechtia polystachya* Griseb were collected from El-Orman garden, Giza, Egypt in April 2010. Leaves of *Podocarpus gracilior* Pilger were collected from El-Zohria garden, Cairo, Egypt in June 2010. Identification of the plants was confirmed by Dr. Trease Labe, lecturer of Taxonomy, Department of Floral and Taxonomy, El-Orman garden, Cairo, Egypt.

#### Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl) was purchased from Sigma-Aldrich Co. (St Louis, MO). All other chemicals, solvents and reagents used in chromatography were of analytical grade. Authentic reference of phenolic compounds were obtained from Phytochemistry Laboratory, Department of Molecular and cell Biology, University of Texas at Austin (Austin, TX) and from Pharmacognosy Department, Faculty of Pharmacy, Helwan University, Helwan, Egypt. Authentic of fatty acids, hydrocarbons and sterols were obtained from Faculty of Agriculture Research Park, Giza, Egypt.

#### Cell line and culture medium

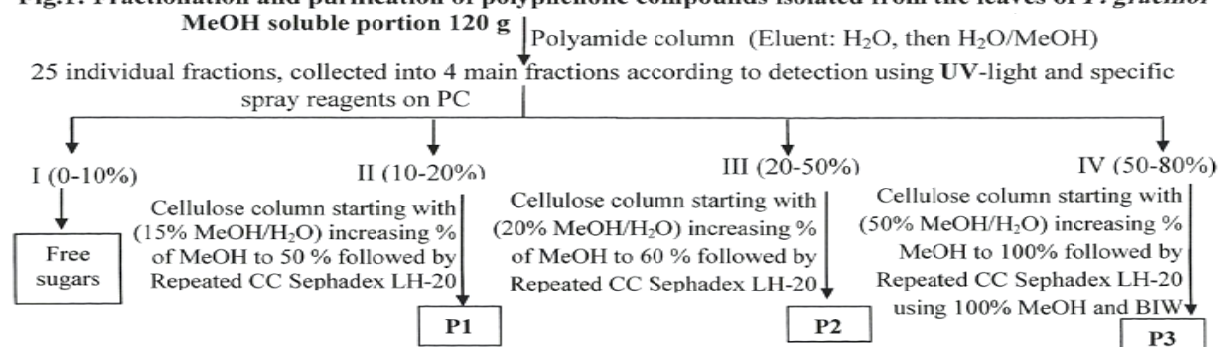
Human breast adenocarcinoma cell line (MCF-7), purchased from ATCC, USA, and was used to evaluate the cytotoxic effect of the tested samples. Cells were routinely cultured in DMEM (Dulbecco's Modified Eagle's Medium), supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/ml penicillin G sodium, 100

units/ml streptomycin sulphate, and 250 ng/ml amphotericin B. Cells were maintained at sub-confluence at 37°C in humidified air containing 5% CO<sub>2</sub>. For sub-culturing, monolayer cells were harvested after trypsin/EDTA treatment at 37°C. Cells were used when confluence had reached 75%. Tested extracts were dissolved in dimethyl sulphoxide (DMSO), and then diluted thousand times in the assay. All cell culture material was obtained from Cambrex BioScience (Copenhagen, Denmark)<sup>[15]</sup>.

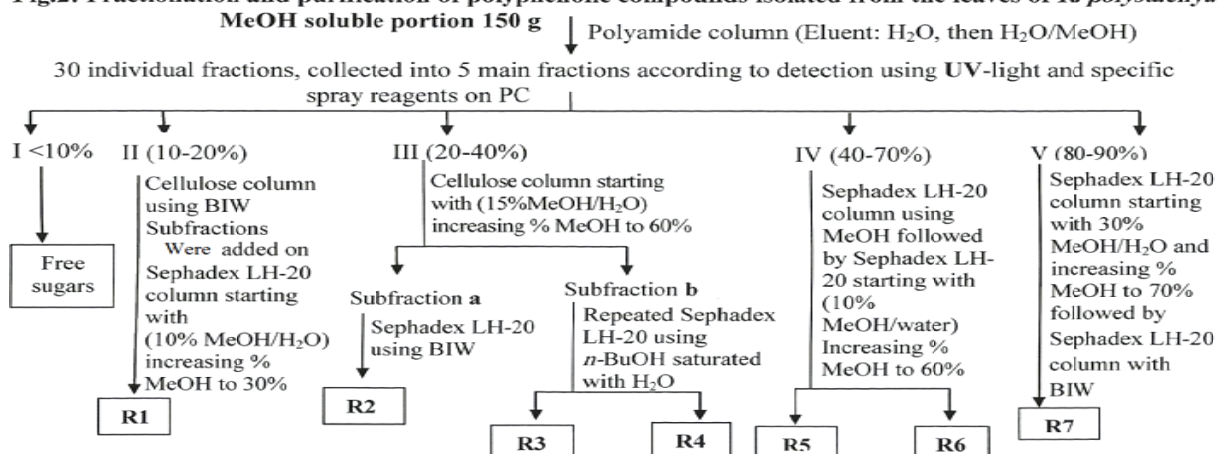
#### Extraction and isolation

Powdered, air-dried leaves of *P. gracilior* (1050 g) and *R. polystachya* (950 g) were separately exhaustively extracted with hot 80% MeOH (5×3 L), under reflux. The dry residues obtained of *P. gracilior* (140 g) and *R. polystachya* (170 g) were extracted with chloroform (3×1 L). The 2D-PC revealed that chloroform soluble portion contained limited polyphenolic contents, while they were concentrated in MeOH soluble portion. The aqueous residues of *P. gracilior* (120 g) and *R. polystachya* (150 g) were fractionated on a polyamide column (Ø 5.5×120 cm) as illustrated in flow charts (Figures 1 and 2). Three compounds were isolated from *P. gracilior* and seven compounds were isolated from *R. polystachya* (Figures 1 and 2). While the chloroform soluble portions concentrated under vacuum (12 g for *P. gracilior* and 15 g for *R. polystachya*) were used for identification of lipoidal matters in both plants. The chloroform extracts were saponified with 10% ethanolic potassium hydroxide under reflux<sup>[16]</sup>. After evaporation of the ethanol, the aqueous solution was extracted with chloroform. The collected chloroform was washed with water and dried over anhydrous sodium sulphate, evaporated and weighted 2.3 g (19%) and 3.5 g (23%) for *P. gracilior* and *R. polystachya* respectively then kept for further study by GLC (USM). The remaining aqueous layer after extraction with chloroform was acidified with 10% hydrochloric acid and the liberated fatty acids were extracted with chloroform and washed with distilled water, dried over anhydrous sodium sulphate, evaporated then weighted 5 g (42%) and 5.5 g (37%) for *P. gracilior* and *R. polystachya* respectively and were kept for further study of TFA according to Vogel method<sup>[17]</sup>.



**Fig.1: Fractionation and purification of polyphenolic compounds isolated from the leaves of *P. gracilior*****Table 1: Solvent systems**

S <sub>1</sub>	n-Butanol – Acetic acid – Water (BAW)	(4:1:5 v/v/v, upper layer)
S <sub>2</sub>	Acetic acid – Water	(15:85 v/v)
S <sub>3</sub>	n-Butanol – Isopropyl alcohol – Water (BIW)	(4:1:5 v/v/v, upper layer)

**Fig.2: Fractionation and purification of polyphenolic compounds isolated from the leaves of *R. polystachya***

### Anti-tumor activity

Cytotoxic activity for the extracts was measured against MCF-7 cells using the MTT Cell Viability Assay. MTT (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) assay is based on the ability of active mitochondrial dehydrogenase enzyme of living cells to cleave the tetrazolium rings of the yellow MTT and form a dark blue insoluble formazan crystals, accumulated within healthy cells. Solubilization of the cells results in the liberation and solubilization formazan crystals. The number of viable cells is directly proportional to the level of soluble formazan dark blue color. The extent of the reduction of MTT was quantified by measuring the absorbance at 570 nm<sup>[15]</sup>. Cells (0.5X10<sup>5</sup> cells/ well), in serum-free media, were plated in a flat bottom 96-well microplate, and treated with 20 µl of different concentrations of the tested samples for 48 hrs at 37°C, in a humidified 5% CO<sub>2</sub> atmosphere. After incubation, media were removed and 40 µl MTT

solution / well were added and incubated for an additional 4 hrs. MTT crystals were dissolved by adding 180 µl of acidified isopropanol/ well and plate was shaken at room temperature, followed by photometric determination of the absorbance at 570 nm using microplate ELISA reader. Triplicate repeats were performed for each concentration and the average was calculated. Data were expressed as the percentage of relative viability compared with the untreated cells compared with the vehicle control, with cytotoxicity indicated by <100% relative viability (Figure 8)<sup>[15]</sup>.

### Antioxidant activity

Free radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation. The method of scavenging DPPH free radicals can be used to evaluate the antioxidant activity of methanol extracts obtained from the leaves of *P. gracilior* and *R. polystachya*. Ethanolic DPPH

(1,1-diphenyl-2-picrylhydrazyl): 0.1 mM DPPH/absolute ethanol. In the presence of an antioxidant radical scavenger, which donates an electron to DPPH, the deep violet color decolorize to the pale yellow non-radical form are which monitored spectrophotometrically at 515 nm<sup>[18]</sup>. In a flat bottom 96 well-micro plates, a total test volume of 200 µl was used. In each well, 20 µl of different concentrations (0-40 µg/ml final concentration) of the tested samples were mixed with 180 µl of ethanolic DPPH and incubated for 30 min at 37°C. Triplicate wells were prepared for each concentration and the average was calculated. Then the absorbance at 515 nm was determined photometrically by micro plate ELISA reader. Concentrations ranging from 0-25 µg/ml standard ascorbic acid solutions were used for plotting a standard calibration curve<sup>[18&19]</sup>.

#### Evaluation of anti-inflammatory activity

Nitrite accumulation was used as an indicator of NO production using a micro plate assay based on the Griess reaction. The Griess reaction is based on a two-step diazotization reaction in which acidified nitrites generate a nitrosating agent that reacts, with sulfanilic acid to form diazonium ion. This ion is then coupled to N-(1-naphthyl) ethylenediamine to produce the chromophoric pink azo-derivative that can be determined spectrophotometrically at 540 nm<sup>[20]</sup>. 40 mg Griess reagent (0.2% naphthylenediamine dihydrochloride, and 2% sulphanilamide in 5% phosphoric acid) was dissolved in 1 ml deionized water. In each well of a flat bottom 96 well- micro plate, 40 µl freshly prepared Griess reagent was mixed with 40 µl cell supernatant after cell treatment with LPS (lipopolysaccharides). 25 µg/ml of the methanol extracts of both *P. gracilior* leaves and *R. polystachya* leaves were separately added and incubated for 48 hrs. The plates were incubated for 10 min in the dark then the absorbances of the mixtures at 540 nm were determined using the micro plate ELISA reader. A standard curve relating NO in µM to the absorbance was constructed (Figure 9), from which the NO level in the cell supernatant was computed by interpolation.

#### Antimicrobial study

Gram positive bacteria (*Staphylococcus aureus* ATCC12600), Gram negative bacteria (*E. coli* ATCC117750), Yeast (*Candida albicans* ATCC26555) and Fungi (*Aspergillus flavus*) were used to test the antimicrobial activity of *P. gracilior* and *R. polystachya* methanolic extracts. The antimicrobial study of the tested methanol extracts was determined by applying modified disc diffusion method<sup>[21]</sup>. The extract residue was diluted with

DMSO at concentration 1:5 w/v, then 20 µl was aseptically transferred onto sterile discs of Whatmann filter paper (5 mm diameter). Standard discs of tetracycline and amphotericin B served as positive controls for antimicrobial activity but filter discs impregnated with 20 µl of solvent (DMSO) were used as negative control. The diameters of the inhibition zones were measured in millimeters by the use of slipping calipers (Table 4).

### 3. Results and discussion:

#### Investigation of polyphenolic contents

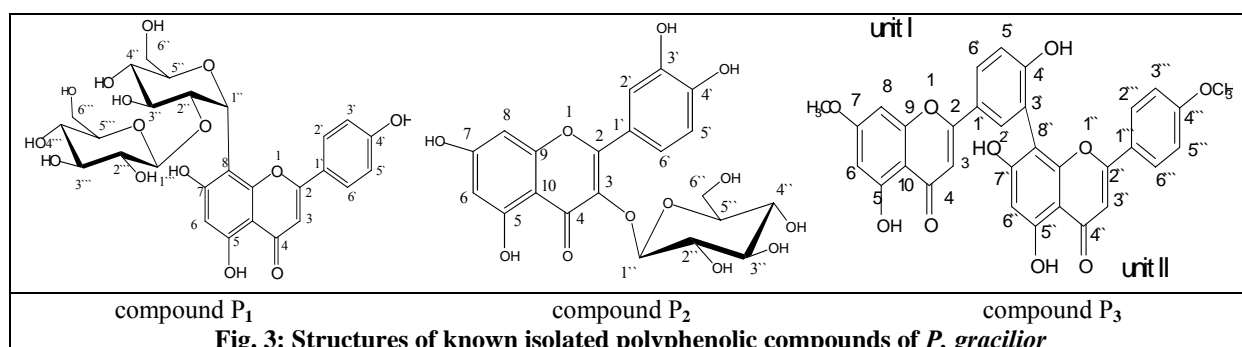
Methanol extracts of *P. gracilior* and *R. polystachya* leaves were separately fractionated on a polyamide column followed by successive separation on cellulose and Sephadex LH-20 columns yielded three and seven compounds in *P. gracilior* and *R. polystachya* leaves extracts respectively (Figures 1 and 2). The isolated pure compounds were identified on the basis of acid hydrolysis, comparative PC, UV, ESI-MS, <sup>1</sup>H-, <sup>13</sup>C-NMR and in some cases 2D-NMR spectroscopic analyses and comparing with previous reported data<sup>[10,12&22-34]</sup>. The known isolated compounds from *P. gracilior* leaves are identified (Figure 3) as P<sub>1</sub>: Apigenin 8-C-β-D-glucopyranosyl-(1''→2'')-O-β-D-glucopyranoside (Vitexin 2''- O-β-D-glucopyranoside) which is isolated for the first time from genus *Podocarpus*. P<sub>2</sub>: Quercetin 3-O-β-D-glucopyranoside (Isoquercetin) which is isolated for the first time from *P. gracilior* but isolated before from *P. fasciculus* bark<sup>[35]</sup> and P<sub>3</sub>: 11'-O-7-dimethoxy amentoflavone (Podocarpusflavone B) which is isolated for the first time from *P. gracilior* but was isolated before from *P. neriifolius*, *P. fasciculus*, *P. fleuryi*, *P. elongatus*<sup>[35-40]</sup>. In the case of *R. polystachya* leaves extract we have isolated seven compounds (Figure 4) which are identified as R<sub>2</sub>: Gallic acid, which is isolated for the first time from genus *Ruprechtia*, while R<sub>3</sub>: Myricetin-3-O-α-L-rhamnopyranoside (Myricetrin), R<sub>4</sub>: Quercetin-3-O-α-L-rhamnopyranoside (Quercetrin) R<sub>5</sub>: Quercetin-3-O-α-L-arabinofuranoside, were isolated before from *R. polystachya*<sup>[10]</sup> as well as R<sub>6</sub>: Quercetin 3-O-β-D-glucopyranoside (Isoquercetin) and R<sub>7</sub>: Quercetin were isolated for the first time from genus *Ruprechtia*. In addition, the new compound R<sub>1</sub>: 4'-O-Galloyl-myricetin-3-O-α-L-rhamnopyranoside was found in *R. polystachya*, this new compound was identified according to the following description (Figure 5).

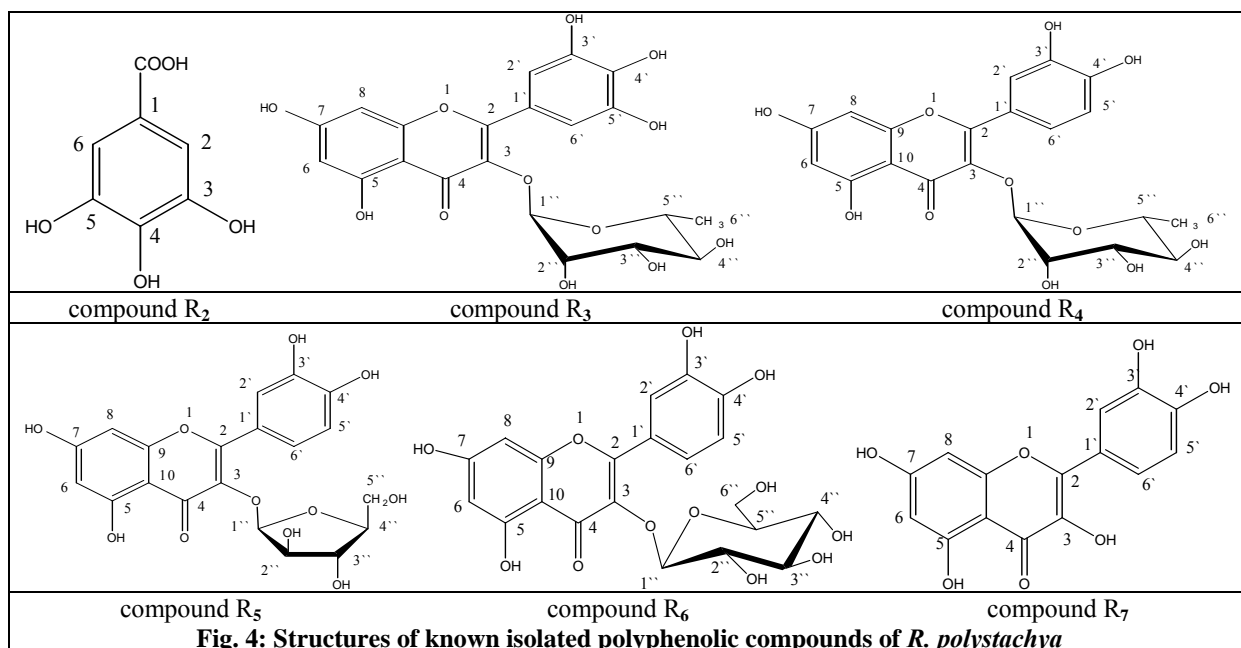
#### Characterization and identification of compound R<sub>1</sub> which is isolated from *R. polystachya*:

Yellow amorphous powder (35 mg). Chromatographic properties, R<sub>f</sub> value: 0.40 (S<sub>1</sub>), 0.62 (S<sub>2</sub>); dark purple spot under UV-light turned to

yellow fluorescence on exposure to ammonia vapors, it gave bluish green color and deep red fluorescence with  $\text{FeCl}_3$  and Naturstoff spray reagents, respectively. UV-spectral data  $\lambda_{\text{max}}$  (nm) (MeOH): 268, 291 (sh), 352; (+NaOMe): 275, 325, 390; (+NaOAc): 278, 329, 387; (+ $\text{AlCl}_3$ ): 277, 427; (+ $\text{AlCl}_3/\text{HCl}$ ): 277, 418. Complete acid hydrolysis gave rhamnose in aqueous phase, myricetin and gallic acid in the organic phase (CoPC).  $^1\text{H}$ NMR spectrum (500 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  ppm 6.88 (2H, s, H-2''/6''), 6.87 (2H, s, H-2'/6'), 6.33 (1H, d,  $J=1.8$  Hz, H-8), 6.16 (1H, d,  $J=1.8$  Hz, H-6), 5.15 (1H, br s, H-1''), 3.94 (1H, br s, H-2''), 3.47-3.13 ( $\text{H}_s$ -3'', 4'', 5'', hidden by  $\text{H}_2\text{O}$  signal), 0.80 (3H, d,  $J=6.0$  Hz, H-6'').  $^{13}\text{C}$ NMR (125 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  ppm 178.27 (C-4), 170.83 (C-7''), 164.74 (C-7), 161.79 (C-5), 158.02 (C-9), 156.93 (C-2), 146.24 (C-3'/5'), 145.87 (C-3''/5''), 138.48 (C-4''), 136.98 (C-4'), 134.77 (C-3), 123.24 (C-1''), 120.12 (C-1'), 109.23 (C-2''/6''), 108.43 (C-2'/6'), 104.53 (C-10), 102.39 (C-1''), 99.22 (C-6), 94.09 (C-8), 71.75 (C-4''), 70.87 (C-3''), 70.86 (C-2''), 70.52 (C-5''), 17.99 ( $\text{CH}_3$ -6''). Negative ESI-MS:  $m/z$  615.29  $[\text{M}-\text{H}]^-$ , 463.17  $[\text{M-galloyl}]^-$ , 317.06  $[\text{Myricetin-H}]^-$ . The UV-spectral data of compound  $\text{R}_1$  showed in the MeOH spectrum two characteristic absorption maxima at  $\lambda_{\text{max}}$  268 nm (band II) and 352 nm (band I), for flavonols. The enhanced UV absorbance of band II at 268 nm in MeOH in comparison with compound  $\text{R}_3$  gave the suggestion of the presence of gallic acid moiety in the structure<sup>[12&41]</sup>. The bathochromic shift and decrease in intensity of band I observed with NaOMe indicated 4'-*O*-substituted compound, while the bathochromic shift in band II with NaOAc was indicative of free 7-OH group. In addition to the strong bathochromic shift in band I remaining after addition of HCl to  $\text{AlCl}_3$  indicated the presence of free 5-OH group and the absence of a free 3-OH group<sup>[12]</sup>. Based on the chromatographic properties and the UV- spectral data, compound  $\text{R}_1$  was expected to be Myricetin 3-*O*-galloyl glycoside. On complete acid hydrolysis,  $\text{R}_1$  gave L- rhamnose in aqueous phase, myricetin and gallic acid in organic phase when compared with standards using PC

(CoPC) suggesting myricetin 3-*O*-galloyl rhamnoside. A supporting evidence achieved from Negative ESI-MS spectrum that showed molecular ion peak  $[\text{M}-\text{H}]^-$  at  $m/z$  615 corresponding to M.wt. 616 and molecular formula  $\text{C}_{28}\text{H}_{24}\text{O}_{16}$  together with the fragment ion peaks at  $m/z$  463 (after the loss of galloyl moiety) and at  $m/z$  317 for  $[\text{Myricetin-H}]^-$  aglycone.  $^1\text{H}$ NMR spectra showed the characteristic three proton resonances of H-2'/6' at  $\delta$  ppm 6.87, H-8 at  $\delta$  ppm 6.33 and H-6 at  $\delta$  ppm 6.16 for myricetin aglycone. The presence of galloyl moiety in the structure was concluded from the proton resonance at  $\delta$  ppm 6.88 (2H,s) assignable to H-2''/6''. The sugar moiety was simply identified as  $\alpha$ -rhamnose from the anomeric proton resonance at 5.15 and doublet signal of  $\text{CH}_3$ -6'' at 0.80 (6.0 Hz)<sup>[12]</sup>. The structure of compound  $\text{R}_1$  was finally confirmed by  $^{13}\text{C}$ NMR spectrum that showed the characteristic signals at  $\delta$  ppm 178.27, 146.24, 108.43 and 134.77 for C-4, C-3'/5', C-2'/6' and C-3 respectively for 3-*O*- substituted myricetin compound. The presence of galloyl moiety was confirmed from the carbon resonance at  $\delta$  ppm 170.83 for the carbonyl carbon (C-7'') and the typical four carbon resonances for the rest of galloyl carbons, the position of esterification by gallic acid was confirmed at OH-4' (not at 3' or 5') from the slight up-field shift C-4' in comparison with compound  $\text{R}_3$ , remaining of symmetrical structure in ring-B and finally from the decreasing of intensity of band I with NaOMe reagent as described before in the UV-spectral results. The  $^{13}\text{C}$ NMR also showed the typical  $^{13}\text{C}$ -resonance for rhamnose moiety characterized by anomeric carbon at  $\delta$  ppm 102.39 and  $\text{CH}_3$ -6'' at 17.99. Stereo-structure of the rhamnoside moiety was established as  $\alpha$ - $^1\text{C}_4$ -pyranose depending on  $\delta$  and  $J$ -values in  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra. Finally compound  $\text{R}_1$  was assigned by comparison with the corresponding published data of structural related compounds<sup>[12,23,41&42]</sup>. Thus compound  $\text{R}_1$  was identified as 4'-*O*-galloyl myricetin-3-*O*- $\alpha$ -L-rhamnopyranoside (4'-*O*-galloyl myricetrin) which has been identified as a new natural compound.



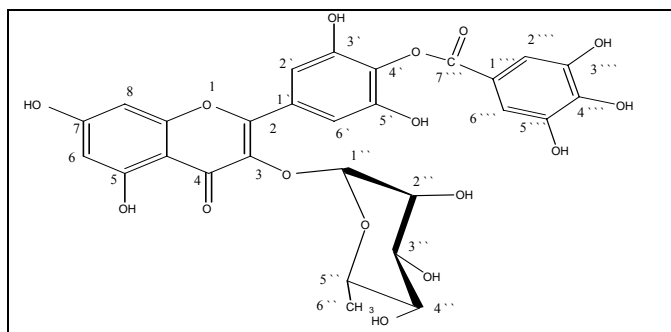


**Fig. 4: Structures of known isolated polyphenolic compounds of *R. polystachya***

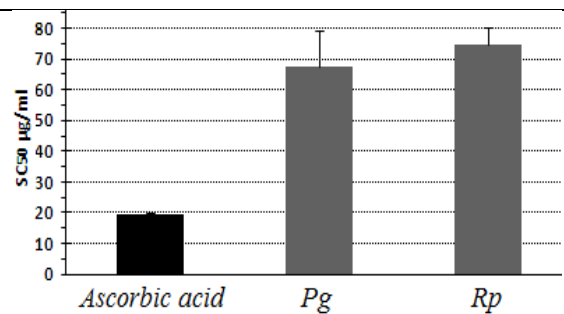
**Investigation of Lipoidal Matter (USM and TFA)**

Identification of hydrocarbons in *P. gracilior* and *R. polystachya* leaves resulted in identification of 19 and 21 compounds respectively (Table 2). n-Nonane (38.91%) and n-Tetracosane (28.74%) represent the major hydrocarbons in *P. gracilior* and *R. polystachya* respectively.  $\beta$ -sitosterol and stigmasterol represent the two identified sterols in

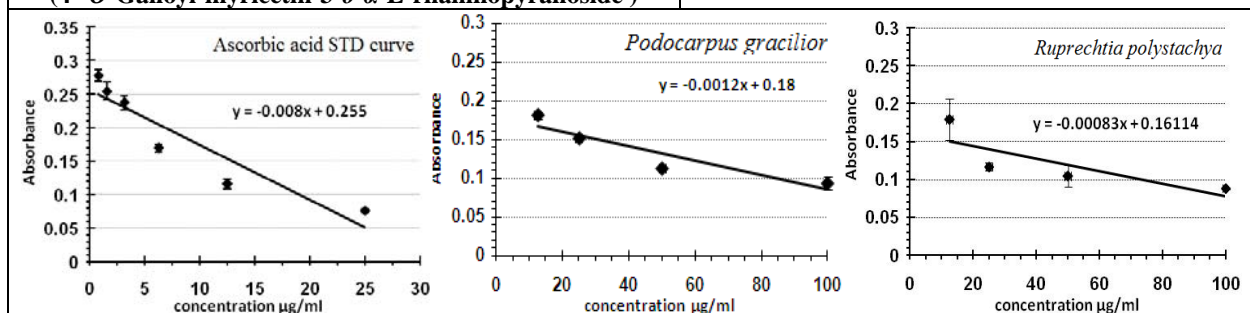
both *P. gracilior* and *R. polystachya*. As shown in table 3 concerning the composition of fatty acids content in *P. gracilior* it could be concluded that the unsaturated fatty acids (51.16%) represented higher percentage than that of saturated ones (38.87%). In the case of *R. polystachya* the saturated fatty acids (70.58%) represented higher percentage than that of unsaturated ones (27.3 %) (Table 3).



**Fig. 5: Compound R<sub>1</sub>, 4'-O-galloyl myricitrin (4'-O-Galloyl-myricetin-3-O- $\alpha$ -L-rhamnopyranoside)**



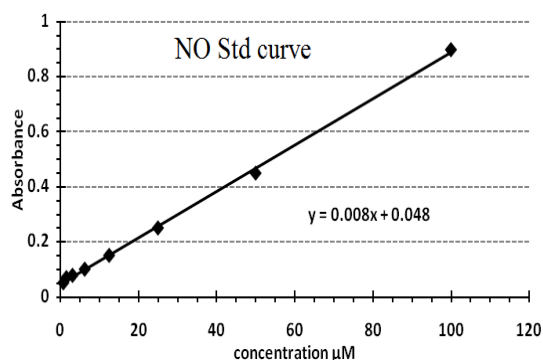
**Fig. 6: Calculated SC<sub>50</sub> of methanol extracts from *Pg* and *Rp* leaves using DPPH radicals**



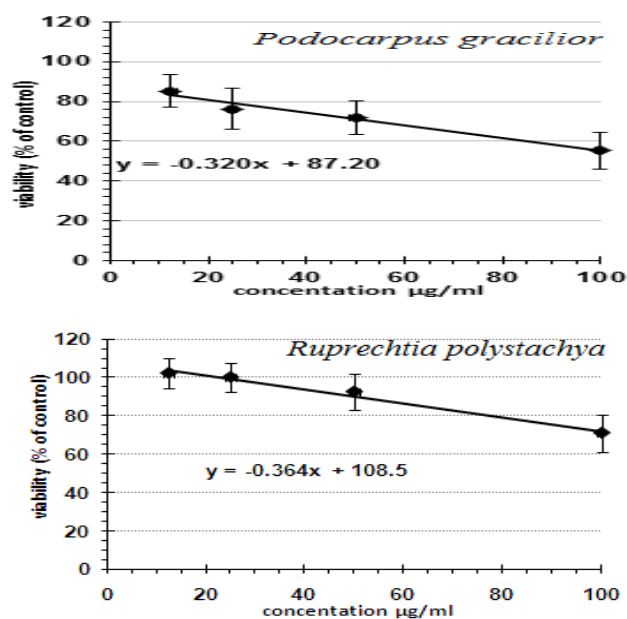
**Fig. 7: Absorbance of various drugs versus concentrations showing different antioxidant activities.**

**Table 2: GLC analysis of USM of *Pg* and *Rp* leaves (as % of total USM)**

Identified compounds	<i>Pg</i>	<i>Rp</i>
n-Nonane (C9)	38.91	1.50
n-Decane (C10)	0.71	0.53
n-Henedecane (C11)	0.84	0.30
n-Dodecane (C12)	2.90	13.57
n-Tridecane (C13)	1.23	1.36
n-Tetradecane (C14)	2.15	2.62
n-Pentadecane (C15)	0.89	7.15
n-Hexadecane (C16)	0.85	1.01
n-Heptadecane(C17)	0.88	0.69
n-Octadecane (C18)	1.51	1.11
n-Nonadecane (C19)	3.17	10.07
n-Eicosane (C20)	2.02	3.35
n-heneicosane (C21)	10.83	0.51
n-docosane (C22)	0.83	0.40
n-Tetracosane (C24)	1.57	28.74
n-pentacosane (C25)	-	0.79
n-hexacosane (C26)	3.67	2.04
n-heptacosane (C27)	1.47	0.64
n-octacosane (C28)	2.92	0.83
n-Nonacosane (C29)	0.37	0.56
n-triacontane (C30)	-	8.07
Stigmasterol	0.79	1.54
$\beta$ -sitosterol	1.05	1.31
Total hydrocarbons	77.71	85.85
Total sterols	1.84	2.85
Unidentified compounds	20.45	11.3

**Fig. 9: A standard curve of sodium nitrite****Table 3: GLC analysis of fatty acid methyl ester of *Pg* and *Rp* leaves (% of fatty acids)**

Identified compounds	<i>Pg</i>	<i>Rp</i>
Isocapric (6:0)	-	9.60
Caprylic (8:0)	-	3.48
Capric (10:0)	1.05	-
Lauric (12:0)	0.35	1.06
Myristic (14:0)	0.43	21.54
Palmitic (16:0)	30.75	25
Hexadecenoic acid (16:1)	0.2	3.40
Margaric (17:0)	0.85	1.32
Stearic (18:0)	4.71	6.30
Octadecenoic acid (18:1)	15.96	7.07
Linoleic (18:2)	-	2.13
Linoelaidic (18:2)	14.44	13.37
Octadecatrienoic acid (18:3)	20.83	1.33
Arachidic (20:0)	0.73	2.30
Saturated fatty acids	38.87	70.60
Unsaturated fatty acids	51.16	27.30
Unidentified compounds	9.97	2.10

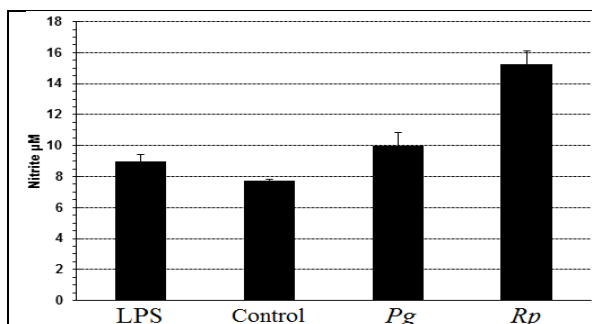
**Fig. 8: Cytotoxic effect of different samples against MCF-7 cells using MTT assay (n=4), data expressed as the mean value of cell viability (% of control)  $\pm$  S.E.**

### Biological study

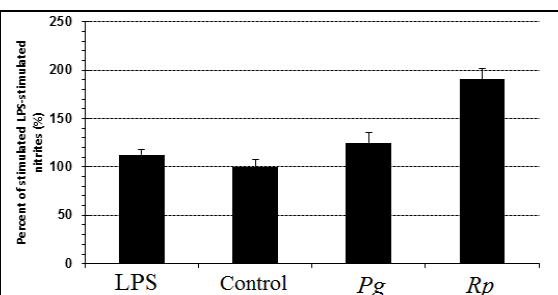
#### Antioxidant activity

The methanol extracts of both *P. gracilior* and *R. polystachya* leaves were proved to exhibit antioxidant scavenging affinity against DPPH as concluded from its  $SC_{50}$  value 67.4  $\mu$ g/ml and 74.8  $\mu$ g/ml

respectively when compared with the standard antioxidant activity of vitamin C; ascorbic acid ( $SC_{50}$  20  $\mu$ g/ml), as shown in figures 6 and 7.



**Fig. 10:** The level of Nitric oxide in RAW 264.7 cells supernatant after the treatment with the samples (25 µg/ml) for 48 hours compared with LPS-treated cells, as measured by Griess assay.



**Fig. 11:** The percentage of stimulation of Nitric oxide in LPS-stimulated RAW 264.7 cells supernatant after the treatment with the samples (25 µg/ml) for 48 hours compared with LPS treated cells, as measured by Griess assay.

#### Anti-tumor activity

Cytotoxic activity of methanol extracts obtained from the leaves of *P. gracilior* and *R. polystachya* were examined using MCF-7 (breast adenocarcinoma cell line). Activity was reported in terms of an  $IC_{50}$ , which is the concentration (µg/ml) necessary to produce 50% inhibition. Using MTT assay, the effect of both plant extracts on the proliferation of MCF-7 cells were studied after 48 hrs of incubation at 37°C. As shown in figure 8 the treatment of MCF-7 cells with the methanol extract from *P. gracilior* showed weak cytotoxic effect against MCF-7 as its calculated  $IC_{50}$  was 116.3 µg/ml while the methanol extract obtained from *R. polystachya* leaves did not show cytotoxic effect as its calculated  $IC_{50}$  was 160.7 µg/ml<sup>[15]</sup>.

#### Evaluation of anti-inflammatory activity using estimation of nitric oxide method

The results indicated that LPS induced nitric

oxide production up to 12.5% of the control, and that both of the tested samples (25 µg/ml) possessed a highly significant stimulatory activity against LPS- induced nitric oxide ( $P < 0.001$ ) to the extent 25 and 90% for both *P. gracilior* and *R. polystachya* extracts respectively compared to the control level, as shown in figures 9-11. Such stimulatory samples may be used in immune-compromised patients to improve their immunity against various infections.

#### The antimicrobial activity

As shown in table 4, the methanol extracts of the leaves of *P. gracilior* and *R. polystachya* exerted marked effect against Gram -ve and Gram +ve bacteria. It showed that the activity on Gram -ve higher than Gram +ve bacteria. Methanol extracts of the leaves of *P. gracilior* and *R. polystachya* showed no antifungal activity.

**Table.4:** Antimicrobial screening of the methanol extracts of the leaves of *Pg* and *Rp* (diameter of inhibition zone measured by mm)

Microorganism	<i>Pg</i>	<i>Rp</i>	TC	AMP
<i>E. coli</i> (G-ve)	15 (50%)	13 (43.3%)	30 (100%)	-
<i>S. aureus</i> (G+ve)	14 (48.3%)	11 (40%)	29 (100%)	-
<i>A. flavus</i> (fungus)	0.0	0.0	-	17
<i>C. albicans</i> (fungus)	0.0	0.0	-	19

TC: Tetracycline, AMP: Amphotericin B, -: No inhibition zone

#### 4. Conclusion:

In conclusion, the methanol extracts of the leaves of *P. gracilior* and *R. polystachya* contain various polyphenolic compounds. Their extracts have significant antioxidant and antimicrobial properties. The tested methanol extracts of *P. gracilior* and *R. polystachya* leaves have

stimulatory activity to nitric oxide release from macrophage cell line (so may be used in immune-compromised patients to improve their immunity against various infections) thus have great potential as a source for natural health products.

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## Prevalence, Types and Risk factors of Non Fatal Injuries among Secondary School Students in Abha City-KSA

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**Abstract:** Background: Injury in developing and transitional economies is an important public health problem. Indeed, injury accounts for 9% of global mortality, with the majority of these injury deaths occurring in lower- and middle-income countries, result in both social and economic loss. Increased availability of motor vehicles is likely to raise the risk of traffic injuries. The inexperience and ongoing neurodevelopment of adolescents might leave them vulnerable to some health risks associated with economic change. The World Health Organization defines injuries as “the physical damage that results when a human body is suddenly subjected to energy in amounts that exceed the threshold of physiological tolerance – or else the result of a lack of one or more vital elements, such as oxygen”. The risk factors for injury vary depending upon its type. The majority of assaults involve younger people. Risks of both unintentional and intentional injury have been shown to be related to socioeconomic status, with those at the greatest risk living in the most deprived areas. Objectives: 1) To calculate injury rates among male secondary school students, 2) To Identify the types of injuries and 3) To Identify possible risk factors associated with injuries. Subjects And Methods: This study followed a cross-sectional design. It was conducted in Abha City. 829 male secondary school students studying at a general governmental secondary school in Abha City were included in the study. Results: The prevalence of non-fatal injuries among male secondary school students was 49%. The Places of the majority of non-fatal injuries were streets (89.9%) while schools and homes were reported by 4.2% and 1.2% of the students. Students who have more than five brothers were at 7.6 folded risk of having non-fatal injuries as compared to those who have no brothers. Students whose family income was between 5001 and 10000 or > 10000 SR/month were at 7 or 13 folded risk for non-fatal accidents, respectively as compared to those whose family income was below or equal 50000 SR/month. Students with history of psychic trouble or chronic diseases were at 4 and 5 folded risks respectively for having non-fatal injury. Smoker students were at double risk for non-fatal accidents than non-smokers. Conclusions: Non-fatal accidents are a prevalent public health problem among male secondary school students in Abha, KSA. Road traffic accidents are the main reported among them. High-family income, large family size, smoking, chronic diseases and psychic troubles were significantly associated with non-fatal injuries.

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**Key words:** Prevalence, Types, Risk, Injuries

### 1. Introduction

Injury in developing and transitional economies is an important public health problem. Indeed, injury accounts for 9% of global mortality, with the majority of these injury deaths occurring in lower- and middle-income countries, result in both social and economic loss (1). Increased availability of motor vehicles is likely to raise the risk of traffic injuries (2). The inexperience and ongoing neurodevelopment of adolescents might leave them vulnerable to some health risks associated with economic change (3).

The World Health Organization defines injuries as “the physical damage that results when a human body is suddenly subjected to energy in amounts that exceed the threshold of physiological tolerance – or else the result of a lack of one or more vital elements, such as oxygen”. Injuries can be classified into two distinct categories, those that are unintentional (e.g., falls) and those that are intentional (e.g., assaults) (4).

Injuries can leave people with serious physical pain, disability and psychological trauma, which may require long-term care by families and health services. Consequently unintentional injuries place a significant economic burden on public services and society as a whole, with loss of work and school days impacting on businesses and individuals (5).

Injuries kill more than 5 million people every year worldwide, accounting for nearly 1 out of every 10 deaths globally. They affect people of all ages and socio-economic status of a nation (6). Unintentional injuries are a leading cause of death among children and young adults. Over 875,000 children <18 years of age die annually in the world as a result of injuries (7). Injuries are the leading cause of long-term disability, and healthcare costs in most industrialized countries (8).

The injury mortality rates of males are twice as high as that of females worldwide.(9) In Qatar, A male to female death ratio of 3.4:1 for all types of

injuries leading to death. This could be due to the fact that boys are more aggressive and adventurous than girls (6). Blum and Nelson-Nmari (10) reported that among leading causes of death in people aged 15–29 years are (unintentional injuries, violence, and suicide) (10).

Patterns of injury change with physical maturity, with young men in particular, incurring trauma mainly from unintentional injuries, violence and traffic accidents (11). Consequently, health profiles change rapidly from early adolescence to young adulthood (3).

Unintentional injuries accounted for around 12,000 deaths in England and Wales in 2007. Self-harm and assaults were also responsible for around 100,000 and 43,000 hospital admissions in England in 2007/08 respectively (12). It was estimated that in UK the cost of interpersonal violence (e.g., assault) alone was £24.4 billion in 2003, with health services incurring a cost of around £2.2 billion (5).

The WHO (13) reported that in the Kingdom of Saudi Arabia, the highest rate for disability-adjusted life years (DALYs) is that for unintentional injuries, being 7.6. It is more than double that of cardiovascular diseases, which ranks the second (3.1).

The most common injuries were falls, road traffic accidents. Males were more affected by injuries than females (14).

Patterns of injury change with physical maturity, with young men in particular, incurring trauma mainly from unintentional injuries, violence and traffic accidents. These injuries have their long-term disabilities of children, economic burden for individuals, and hardship for survivors and family care-givers (15).

The risk factors for injury vary depending upon its type. The majority of assaults involve younger people (16). Furthermore, both children and older people experience higher rates of pedestrian road traffic accidents than other age groups. Risks of both unintentional and intentional injury have been shown to be related to socioeconomic status, with those at the greatest risk living in the most deprived areas (17). The etiology of injury involves a complex interplay between human and environmental factors. Patterns of injury vary according to demographic factors including age, sex, residence and socioeconomic status, all of which are difficult to modify (18). Adolescence, in particular, is an important period because it is the time when young people are experimenting with, and also establishing, their lifestyle, attitudes, concepts, beliefs and habits that may have long-term influences on their health (19).

The rapid social, physical and mental development occurring during adolescence is important as many health behaviours, which become

manifest in adulthood, have their origins during younger years (20). A number of theories offer different explanations for the relationship between development and risk-taking among adolescent. For example, biologically-based theories attribute risk-taking behaviour to genetic predispositions and hormonal changes mediated through pubertal timing (21).

#### **Objectives:**

To calculate injury rates among male secondary school students

To identify the types of injuries

To identify possible risk factors associated with injuries.

#### **2. Subjects And Methods:**

This study followed a cross-sectional design. It was conducted in Abha City, which is the capital of Aseer Region in Saudi Arabia.

We used a multi-stage sampling method to obtain a representative sample. We used school and grade as stratum, and then selected classes by cluster sampling in each stratum. In total, 829 male secondary school students studying at a general governmental secondary school in Abha City were included in the study out of 900 invited to participate in the study with a response rate of 92.1%.

This study concentrated on male secondary school students since males are significantly more vulnerable to injuries than females.

#### **Operational Injury Definition:**

The injury in this study was defined as any injury meeting at least one of the following criteria: (1) an injury for which the student received medical treatment at the school nurse's office, (2) an injury for which the student received emergency medical care from a doctor at a hospital or a private medical office, (3) an injury for which the student received first aid from his/her parent, or (4) an injury that was not treated but caused the student to miss a half day or more of school or regular activities.

A self-administered questionnaire including Personal characteristic of student: Age, family size, birth order, scholastic year, nationality and parent's education, father's job etc. And Variables related to injuries: occurrence of any injury within the past 12 months, type (intentional, unintentional), mechanism (assault, fire/hot exposure, fall, road traffic accident, drowning, poisoning, etc.); site (home, school, outdoor) etc. was used to collect data which was analyzed using SPSS version 18.

Prior to data collection, students were fully informed about the objectives of the study. Each student had the right to accept or refuse participation in the study. Students were notified that they do not have to declare their names on the data collection

sheet. The collected data was kept confidential and will be used only for research purposes.

**3. Results:**

The study included 829 secondary school students out of 900 invited to participate in the study with a response rate of 92.1%. Their socio-demographic characteristics are presented in table (1). Their ages ranged between 15 and 22 years with a mean of 17.5±1.2 years. Second-year (science) and third year (science) students present 44.1% and 26.8% of the participants. Most of the students were Saudi (76.4%). Most of the students reported from one to five brothers (77.9%) and sisters (83.4%). First birth order was found among 23.5% of the students while second and third birth orders were reported among 43.9%. Fathers of more than half of the students had university graduation (50.7%) and mothers of 40% of them had university graduation. The fathers of 22.1% of the students were employees and of 19% were military or police persons. More than half of the students had private houses (53.8%) and more than 5 rooms in the house (60.5%). The family income was more than 10000 in 45.4% of the students.

**Table 1:** Socio-demographic characteristics of secondary school students (n=829).

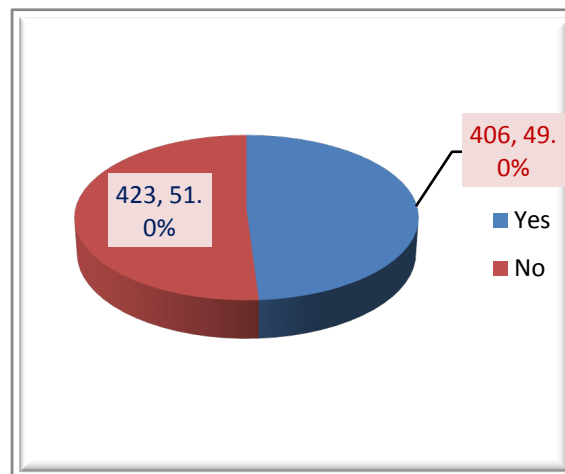
Characteristics	No.	%
<b>Age in years</b>		
≤17	418	50.4
>17	411	49.6
<b>Range (years)</b>	15-22	
<b>Mean±SD (years)</b>	17.5±1.2	
<b>Class</b>		
1 <sup>st</sup>	151	18.2
2 <sup>nd</sup> -Science	366	44.1
2 <sup>nd</sup> -Literature	12	1.4
3 <sup>rd</sup> -Science	222	26.8
3 <sup>rd</sup> -Literature	78	9.4
<b>Nationality</b>		
Saudi	633	76.4
Non-Saudi	196	23.6
<b>Number of bothers</b>		
none	26	3.1
1-5	646	77.9
>5	157	19.0
<b>Number of sisters</b>		
none	98	11.8
1-5	691	83.4
>5	40	4.8
<b>Birth order</b>		
1 <sup>st</sup>	195	23.5
2 <sup>nd</sup> - 3 <sup>rd</sup>	364	43.9
>3 <sup>rd</sup>	270	32.6

**Table 2:** Socio-demographic characteristics of secondary school students (n=829). (cont.)

Characteristics	No.	%
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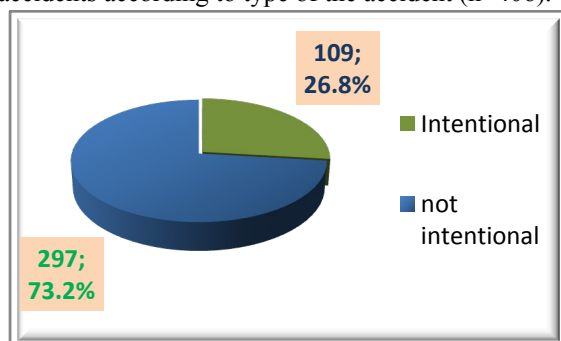
<b>Father's education</b>		
Illiterate	31	3.7
Primary	90	10.9
Intermediate	85	10.3
Secondary	203	24.5
University	420	50.7
<b>Mother's education</b>		
Illiterate	129	15.6
Primary	143	17.2
Intermediate	95	11.5
Secondary	130	15.7
University	332	40.0
<b>Father's job (819)</b>		
Manual worker	79	9.7
Military and police	156	19.0
Teacher	99	12.1
Employee	181	22.1
Physician	73	8.9
Engineer	40	4.9
Retired	122	14.9
Business	69	8.4
<b>Type of residence</b>		
Rent	383	46.2
Private	446	53.8
<b>Income ( in SR/month) (819)</b>		
≤5000	146	17.8
5001-10000	301	36.8
>10000	372	45.4

The prevalence of non-fatal injuries among male secondary school students was 49% as shown in Figure (1),



Most of these injuries were not intentional (73.2%) as displayed in figure (2).

Figure (2): Distribution of students with history of accidents according to type of the accident (n=406).



Regarding types of accidents, 65.4% were car accidents, 14.7% were injuries during playing and 10.1% were stabbing wounds. Suicidal attacks were reported by 7.6% of the students. Figure (3)

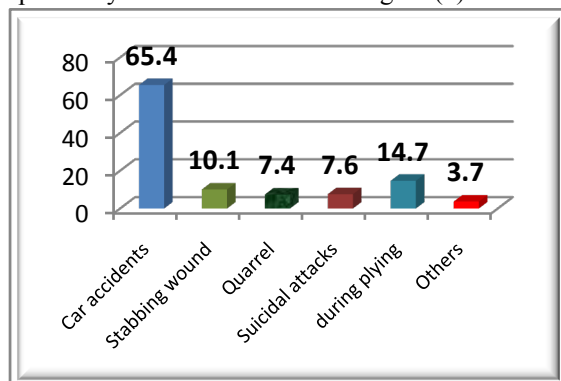


Figure (3): Causes of non-fatal injuries among male secondary school students, Abha city (n=406).

The Places of the majority of non-fatal injuries were streets (89.9%) while schools and homes were reported by 4.2% and 1.2% of the students as shown in figure (4).

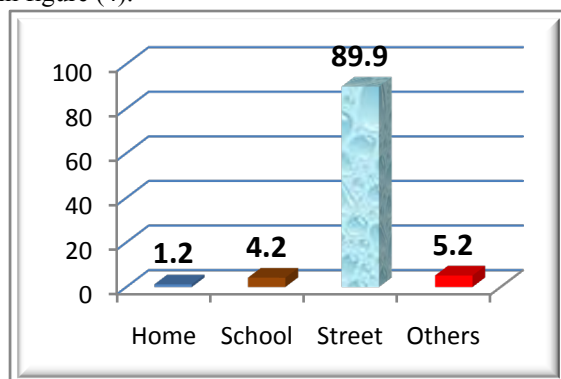


Figure (4): Place of injuries among male secondary school students, Abha city (n=406).

Of students reported non-fatal accidents, 247 (60.8%) had injured during accidents. The results of

injury were wound (63.2%), fracture (17%) or both (19.8%). The place of emergency care was hospital in most of injured cases (80.6%) while it was home or school among 10.5% and 3.6% of the injured students, respectively

44.2% of the injured students stayed one day or less in the hospital while 31.2% of them stayed more than 10 days.

#### Factors associated with non-fatal injuries:

Non-fatal injuries were reported among 58.9% of students aged over 17 years compared to 39.2% among those aged 17 years or less. This difference was statistically significant,  $p < 0.001$ . It was reported among 61.6% of Saudi students compared to only 8.2% among non-Saudi students. This difference was statistically significant,  $p < 0.001$ . Also, it was reported among 59.2% of students who have more than 5 brothers compared to only 15.4% among those having no brothers. This difference was statistically significant,  $p < 0.001$ . 36.9% of students whose fathers are teachers and among 60.9% of students whose fathers are military persons or working in police had not fatal injuries compared to only 24.1% among those whose fathers are manual workers and among 30% of students whose fathers are engineers. This difference was statistically significant,  $p < 0.001$ . Injuries were not significantly associated with student's birth order. Non-fatal injuries were reported among 56.2% of students who fathers are university graduated and among 20% of students whose fathers are primary school educated. This difference was statistically significant,  $p < 0.001$ . Similarly, non-fatal injuries were reported among 52.1% of students who mothers are university graduated and among 34.9% of students whose mothers are illiterate. This difference was statistically significant,  $p = 0.001$ . 71.2% of students whose family income was  $> 10,000$  SR/month had not fatal injuries compared to only 11% among students whose family income was 5,000 or less SR/month. This difference was statistically significant,  $p < 0.001$ .

As regard to the association between type of residence and non-fatal accidents non-fatal injuries were reported among 74.4% of students who has private house compared to only 19.3% among those who has rent house. This difference was statistically significant,  $p < 0.001$ . Non-fatal injuries were reported among 51.8% of students whose family has private car compared to only 17.6% among those whose family has no private car. This difference was statistically significant,  $p < 0.001$ . It also was reported among 85% of students who has nervousness and psychic trouble history compared to 33.2% among those who has no such history. This difference was statistically significant,  $p < 0.001$ . Non-fatal injuries

also were reported among all diabetic and epileptic students (100%) compared to 44.3% among those who has no history of any chronic disease. This difference was statistically significant,  $p < 0.001$ . Non-fatal injuries were reported among 66.7% of students with both of hearing/visual weakness compared to 48.4% among those who has no audio-visual problems. This difference was statistically significant,  $p = 0.024$ . Injuries were reported among 77.5% of smoking students compared to only 40.6% among non-smokers. This difference was statistically significant,  $p < 0.001$ .

**Table 3:** Risk factors for non-fatal injuries among male secondary school students, Abha: Multivariate logistic regression analysis.

Risk factors	OR	95% CI
Number of brothers		
None ®	1.0	
1-5	2.9	0.79-7.11
>5	7.6	1.66-18.31*
Family income ®		
≤5000 (n=146)	1.0	
5001-10,000 (n=301)	6.8	1.62-11.02
>10000 (n=372)	13.4	3.2-22.26
History of psychic trouble		
No ®	1.0	
Yes	3.9	1.29-9.32
History of chronic diseases		
No ®	1.0	
Yes	4.6	2.38-19.25
History of smoking		
No ®	1.0	
Yes	2.1	1.05-4.21

® Reference category

In the multivariate analysis, students who have more than five brothers were at 7.6 folded risk of having non-fatal injuries as compared to those who have no brothers. Students whose family income was between 5001 and 10000 or > 10000 SR/month were at 7 or 13 folded risk for non-fatal accidents, respectively as compared to those whose family income was below or equal 50000 SR/month. Students with history of psychic trouble or chronic diseases were at 4 and 5 folded risks respectively for having non-fatal injury. Smoker students were at double risk for non-fatal accidents than non-smokers. (Table 3)

#### 4. Discussion:

Compared with a rate of 49 non-fatal injuries per 100 adolescents aged 15–22 in our survey, there were 22.1% injuries per 100 children aged 0–17 in USA (22), 11.2 injuries per 100 children aged 0–11 and 17.1 injuries per 100 children aged 12–21 in the US, according to the 1997 NHIS (23). However,

many of the potential risk factors assessed in our study were not assessed in the NHIS. The higher prevalence reported in our study could be attributed to the facts that the present study was conducted on only males and in the age of middle to late adolescents. In Qatar, Bender *et al.* (6) reported a male to female death ratio of 3.4:1 for all types of injuries. This could be due to the fact that boys are more aggressive and adventurous than girls. Similarly, in Riyadh, Gad *et al.* (14) reported 22.2% of children and early adolescents having had injuries in the previous 12 months. Males were more affected by injuries than females (26% vs. 18%).

In the current study, suicidal attacks represent 7.6 of non-fatal injuries among male adolescents. In Western world, a prevalence of 21.2% had been reported by Ross and Heath (61). Islamic regulations prohibit suicidal attacks; is the explanation for the difference between the two prevalence.

Road traffic injuries are a major cause of death and disability globally, with a disproportionate number occurring in developing countries (24,25). In many developed countries, injuries are now the leading cause of death among children and young adults (26). In accordance with that, the present study revealed that car accidents were reported by 65.4% of students with non-fatal accidents (representing a prevalence of 32% among all male students). In Malaysia, the number of motor vehicle injury cases was gradually increased with age and reached its maximum that was 31.0% among the adolescents (27). In Riyadh, Gad *et al.* reported a prevalence of road traffic accidents of 15% among children and early adolescents. This difference could be attributed to difference in age group between the present study and that of Gad *et al.* (14).

Some researchers found that socioeconomic status (SES) was an important risk factor for injuries (28, 29). In our study, we found that non fatal injuries were more among students with higher family income compared to students whose family income was less. Increased availability of motor vehicles is likely to raise the risk of traffic injuries (2). While a study in China showed that low socioeconomic standard students with large family size had a higher risk of injury than high socioeconomic standard students and that socioeconomic background has a major impact on injury risk (30). In our study, students who have more than five brothers were at 7.6 folded risk of having non-fatal injuries as compared to those who have no brothers.

In this study, non-fatal injuries were reported more among students whose mothers are university graduated than among students whose mothers are illiterate. Two other studies indicated that children of unemployed mothers were at greater risk (31,32).

In this study, current smoking, was associated with non fatal injuries These findings resonate with a study among South African school adolescents where, among boys, smoking and drunkenness were found to be predictors for injuries (33),

It is proposed that a number of personality factors may influence a personal risk of becoming injured. Results from the present study showed that injured students had a significantly higher level of psychic troubles in comparison to non-injured students. These results are consistent with Andreas (2010) findings which found a relationship between injury and somatic trait anxiety (34).

In this study, Non-fatal injuries were reported more among students with both of hearing/visual weakness compared to those who has no audio-visual problems. Several studies in the USA, Europe and Australia have reported details on the determinants of injuries at home. Having poor eyesight have been found to be significant predictors of injury-causing falls (35).

In the current study, students with history of chronic diseases were at 5 folded risks for having non-fatal injury and this supports the results of a study of eight countries in Europe. This latter study followed epileptic cases and matched non-epileptic controls for nearly 17,500 person-months, and found that those with epilepsy reported more domestic accidents, more hospitalization and more medical complications compared with the controls (36). Another study showed that Patients with epilepsy or diabetes mellitus have slightly increased risks of traffic accidents as compared with unaffected persons (37).

### Conclusions:

Non-fatal accidents are a prevalent public health problem among male secondary school students in Abha, KSA. Road traffic accidents are the main reported among them. High-family income, large family size, smoking, chronic diseases and psychic troubles were significantly associated with non-fatal injuries.

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## Biochemical and histological studies on the effect of the Patulin mycotoxin on male rats' liver and treatment by crude venom extracted from jelly fish

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**Abstract:** Patulin mycotoxin on some biochemical parameters and histological changes on male rats' liver and effect of crude venom extracted from jelly fish *Cassiopea Andromeda* as a treatment. 50 Inbreeding weanling white male wistar lewis rats were divided randomly into 5 groups. Control group was gavage fed daily with distilled water; three treated groups were gavage fed daily dose with Patulin (0.2 mg/kg b.w.) for one, two and three weeks respectively. The last group was treated by Patulin for one week then injected intraperitoneally with single dose of crude venom (1.78 mg/20 g b.w.) for 24 hours according to LD<sub>50</sub>. Level of (AST) and (GGT) were increased significantly in serum of all treated groups compared with control group but level of (ALT) was increased significantly and gradually in all treated groups, the concentration of ferritin was decreased significantly in treated three after three weeks only. Histopathological changes of rat liver coincided with biochemical changes. In conclusion, oral exposures of Patulin indicate that hepatic alteration was produced in manner related to dose duration and crude venom may used as new therapeutic approach to detoxify hepatocytes from Patulin.

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**Keywords:** mycotoxin, patulin, liver, rat, Jelly fish crude venom.

### 1. Introduction

Huge quantities of food are wasted every year because they are invaded by toxic fungi or contaminated by fungal metabolic products. Over the past 15-20 years, toxic mold exposure has become hazardous and frequent.<sup>1</sup> Mycotoxins are secondary metabolites produced by certain filamentous fungi, which can be produced in foods as a result of fungal growth.<sup>2</sup> Fruits and vegetables are an important part of a balanced diet, but they may be colonized by moulds that produce mycotoxins<sup>3</sup>. In fruit juice, Patulin is considered to be the most important and is produced by several species of fungi including *Penicillium expansum*.<sup>4</sup>

Many experimental studies have been carried out using crude venom extracted from jelly fish for the treatment of animal disease. Venoms are complex mixtures of many different components, whereas a toxin is a single pure compound. Toxins found within venoms often have novel, highly specific activities that have the way for the design of therapeutically useful molecules based upon the structural information obtained.<sup>5</sup> The toxins of coelenterates have been detected to include bradykinnin and related polypeptides which have a kinin like action.<sup>6</sup> BK have effective role in liver regeneration<sup>7,8</sup> and

enhanced prostaglandin synthesis<sup>9</sup> which in turn improved hepatic function.<sup>10</sup>

The work plan involves testing experimental animals for the influence of patulin mycotoxin on liver enzymes, some tumor markers, carbohydrate metabolism as reflected on liver function and structure. Additional attentions are given to crude venom extracted from jelly fish as treatment.

### 2. Materials and methods

Fifty white male Wistar Lewis rats each weighs (55-65 g) were obtained from the animal facility of King Fahd Medical Research Center, King Abdul-Aziz University, Jeddah, Saudi Arabia. The animals were conditioned at room temperature and commercial balanced diet and tap water was provided throughout the experiment. Animals were divided randomly into five groups (10 rats each in two cages) and were subjected to the following schedule of treatments: Control group was gavage fed daily with distilled water; three other treated groups were gavage fed daily dose with Patulin (0.2 mg/kg b. w.) for one, two and three weeks respectively. The last fifth group was treated by Patulin as treated one for one week then injected intraperitoneally with a single dose of crude venom from jelly fish (1.78 mg/ 20 g b.w.) for 24 hours according to LD<sub>50</sub>. Blood samples



were collected from the orbital sinus and stored at -20°C until analysis was performed. Sera were used for the measurement of AST, ALT, GGT, TNF, ferritin and glucose. The animal was decapitated and the abdomen was opened. The liver was excised and washed in sterile saline solution. Small parts were taken for histological studies and other parts of liver were stored at -20°C for glycogen analysis.

### 3. Results

The biochemical and histological changes were studied in blood and liver of male Wistar Lewis rats as a result of administration of a daily dose of Patulin (0.2 mg/kg b. w.) and observed over a period of one (T1), two (T2) and three (T3) weeks compared to the control group. In addition to treated four (T4) using single dose intraperitoneally of crude venom extracted from jelly fish (1.78 mg/ 20 g b. w.) for 24 hours after treated with Patulin for one week (Tables 1, 2).

**Table 1:** Effect of Patulin (0.2 mg/Kg b. w.) on Some Biochemical Parameters of Male Rats.

Groups Parameters	Control	T1	T2	T3
AST U/L	35.146 ± 0.42	70.656±0.79**	72.404±0.87**	65.596±0.41**
ALT U/L	41.073 ± 0.68	45.341 ± 0.97**	39.393 ± 0.71 N.S	40.528 ± 0.99 N.S
GGT U/L	8.203 ± 0.21	9.834 ± 0.41**	9.539 ± 0.25**	9.899 ± 0.22**
TNF Pg/ml	38.027 ± 0.97	53.925 ± 0.81**	60.680 ± 0.35 **	93.588 ± 0.98 **
Ferritin µg/ml	1.617 ± 0.03	1.605 ± 0.06 N.S	1.539 ± 0.08 N.S	1.048 ± 0.06 **
Glucose mmol/l	3.495 ± 0.11	3.356 ± 0.14 N.S	4.127 ± 0.06 **	3.974 ± 0.08 **

Data are expressed as mean ± SE. Number of sample in each group is 10. T1, T2, T3 = Treated group with Patulin  
Significant change in comparison between groups:

\*\* Highly significant ( $P \leq 0.01$ ) \* Significant ( $0.01 < P \leq 0.05$ ) N.S Non significant ( $P > 0.05$ )

**Table 2:** Effect of Crude Venom (1.78 mg/ 20 g b. w.) on Some Biochemical Parameters in Male Rats after Patulin (0.2 mg/kg b. w.) Hepatotoxicity

Parameter	Control	T 1	T 4
AST U/L	35.146 ± 0.42	70.656±0.79	51.253±0.71
			**
		●●	●●
ALT U/L	41.073 ± 0.68	45.341 ± 0.97	41.232 ± 0.84
			**
		●●	N.S
GGT U/L	8.203 ± 0.21	9.834 ± 0.41	8.819 ± 0.24
			*
		●●	N.S
TNF Pg/ml	38.027 ± 0.97	53.925 ± 0.81	46.445 ± 0.67
			**
		●●	●●
Ferritin µg/ml	1.617 ± 0.03	1.605 ± 0.06	1.607 ± 0.03
			N.S
		N.S	N.S
Glucose mmol/l	3.495 ± 0.11	3.356 ± 0.14	3.217 ± 0.08
			N.S
		N.S	N.S
Glycogen µg/µl	0.052 ± 0.01	0.038 ± 0.004	0.037 ± 0.003
			N.S
		N.S	N.S

Data are expressed as mean ± SE. Number of sample in each group is 10.

T1 Treated group with Patulin T4 Treated group with Patulin + crude venom of jelly fish

Significant change in comparison between groups:

\*\*, ●● Highly significant ( $P \leq 0.01$ ) \* Significant ( $0.01 < P \leq 0.05$ ) N.S Non significant ( $P > 0.05$ )

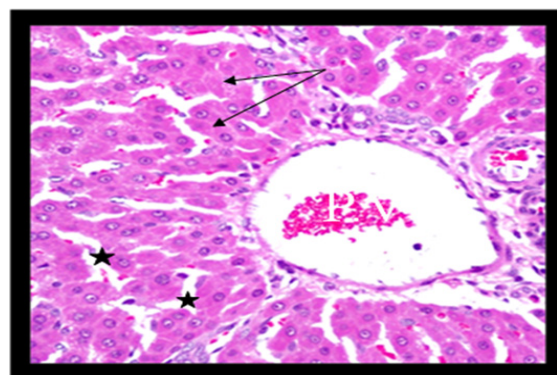
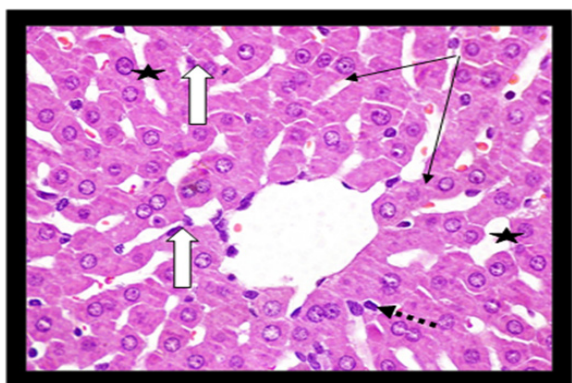
First significant change as compared with T1, Second significant change as compared with control.

### Histological results:

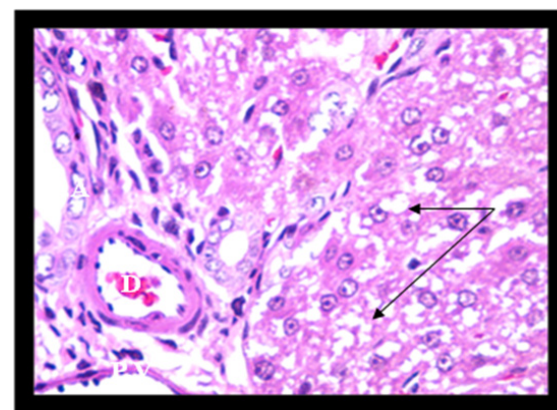
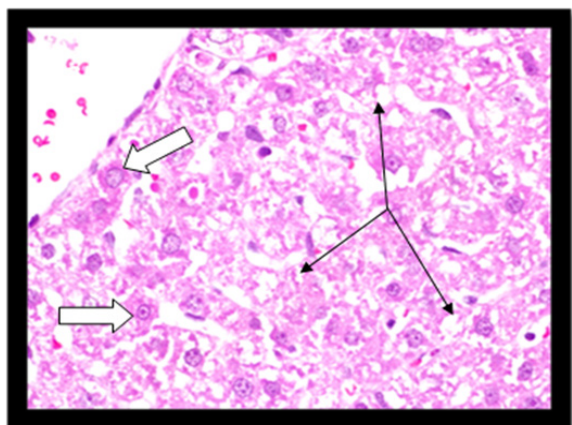
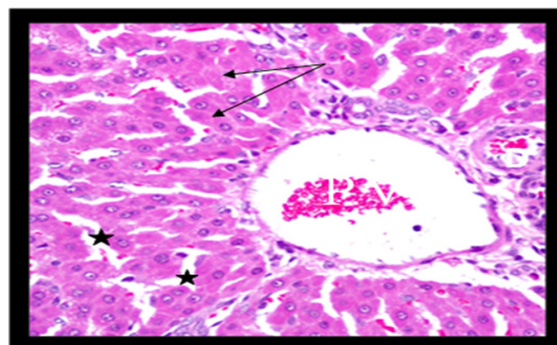
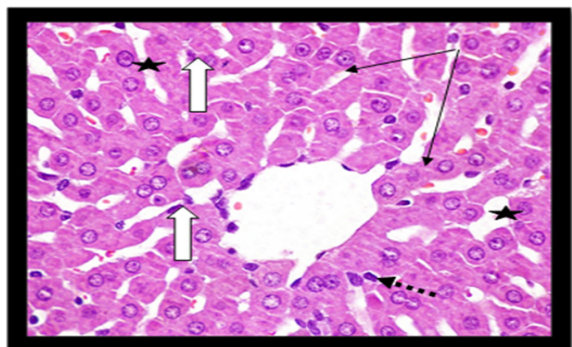
The effect of Patulin on liver parenchyma varies in different weeks. In animal group of one

week (T1) (**Fig. 2**), there was evident hepatocytes vacuolation and damage of cellular membrane (necrosis), while in group of two weeks (T2) (**Fig. 3**) the histological changes were less compared to those of one week (T1). Instead of vacuolation, scattered apoptotic cells were observed in groups of two weeks (T2) and three weeks (T3) (**Fig. 4**). The proliferation of bile ducts in group of three weeks (T3) was described to be associated with hepatic metabolic

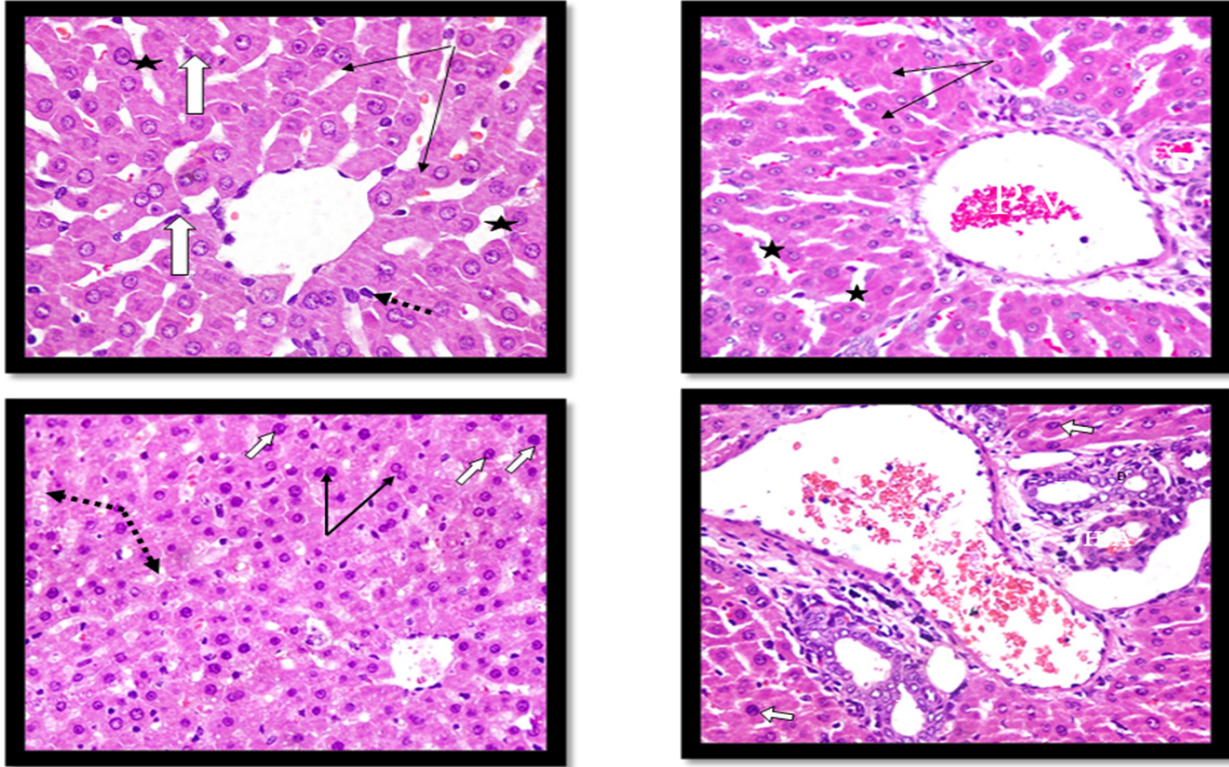
disturbance or toxic exposure.<sup>11</sup> The nuclei showed marked variation in size, Pyknosis (small dark nuclei), karyomegally (large sized) and karyolysis (degeneration) were observed. The enlarged nuclei was observed mainly in animals given Patulin for two weeks (T2) and three weeks (T3) and also in jelly fish treated group (T4) (**Fig. 5**) which may as a result of hepatocytes toxicity.



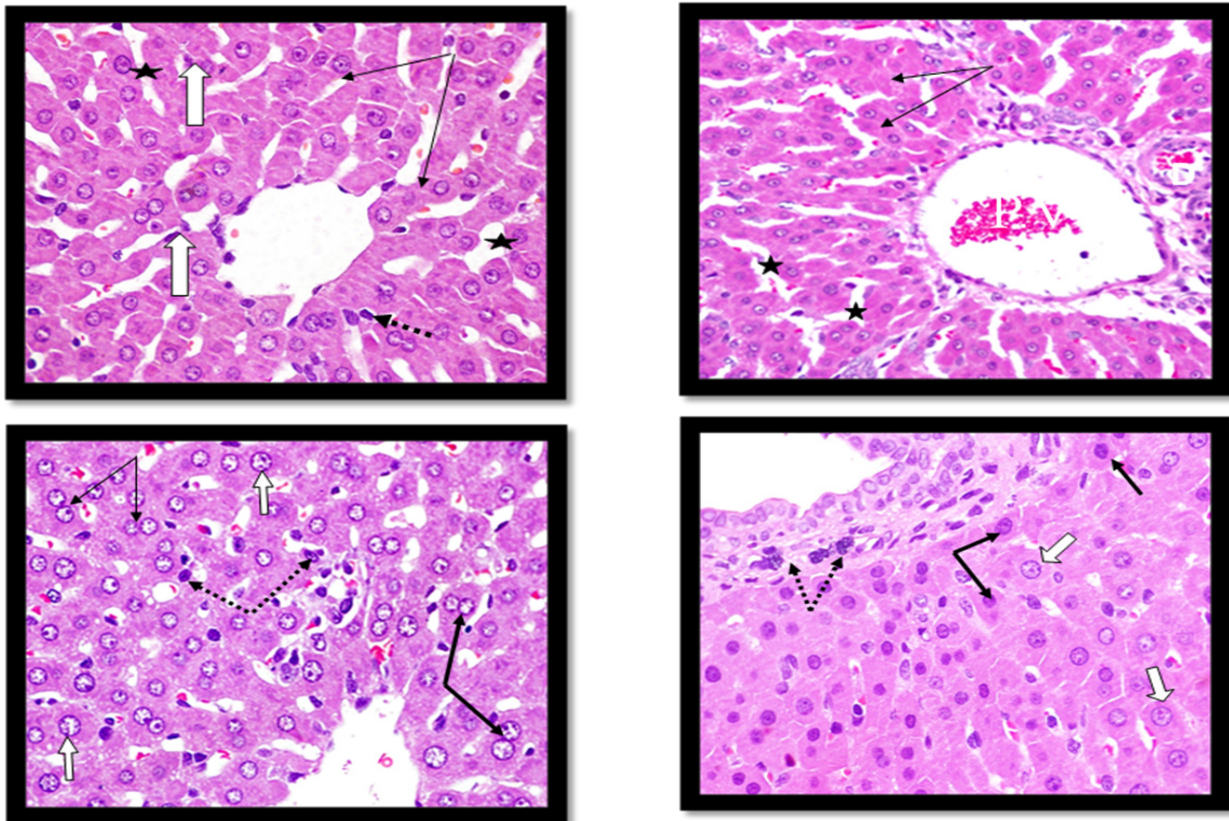
**Fig. 1:** Normal Hepatocytes



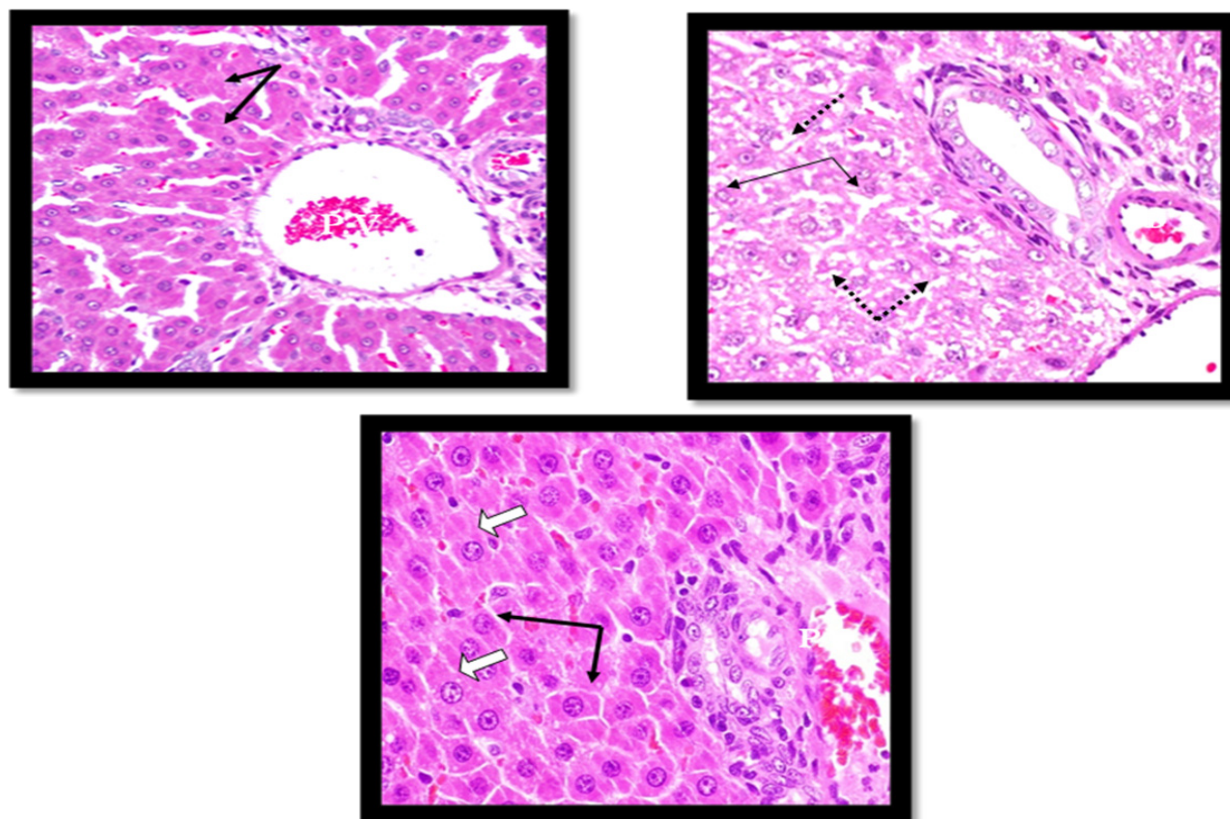
**Fig. 2:** Normal Hepatocytes & The effect of Patulin on liver parenchyma after one week (T1 Hepatocytes)



**Fig. 3:** Normal Hepatocytes & The effect of Patulin on liver parenchyma after two weeks (T2 Hepatocytes)



**Fig. 4:** Normal Hepatocytes & The effect of Patulin on liver parenchyma after three weeks (T3 Hepatocytes)



**Fig. 5:** Normal Hepatocytes & The effect of Patulin on liver parenchyma after one week (T1 Hepatocytes) & The effect of Patulin and crude venom on liver parenchyma after one week (T4 Hepatocytes)

### Discussion

From the data in table (3), the level of AST and ALT were increased than the control group after administration of Patulin for one week (T1), serum transaminases levels in normal subjects are low and liver tissues is rich in both transaminases. So, the

leakage of them into the serum is an indicative of hepatotoxicity (Westhuizen et al., 2001). El-Sawi et al., 2000 reported that the presence of nonfunctional plasma enzymes at levels elevated above normal values suggested an increase rate of tissue destruction.

**Table 3:** Effect of Patulin (0.2 mg/Kg B.W) on Some Biochemical Parameters of Male Rats.

Parameters \ Groups	Control	T1	T2	T3
AST U/L	35.146 ± 0.42	70.656±0.79 **	72.404±0.87 **	65.596±0.41 **
ALT U/L	41.073 ± 0.68	45.341 ± 0.97 **	39.393 ± 0.71 N.S	40.528 ± 0.99 N.S
GGT U/L	8.203 ± 0.21	9.834 ± 0.41 **	9.539 ± 0.25 **	9.899 ± 0.22 **
TNF-α Pg/ml	38.027 ± 0.97	53.925 ± 0.81 **	60.680 ± 0.35 **	93.588 ± 0.98 **
Ferritin µg/ml	1.617 ± 0.03	1.605 ± 0.06 N.S	1.539 ± 0.08 N.S	1.048 ± 0.06 **
Glucose mmol/l	3.495 ± 0.11	3.356 ± 0.14 N.S	4.127 ± 0.06 **	3.974 ± 0.08 **
Glycogen µg/µl	0.052 ± 0.01	0.038 ± 0.004 N.S	0.038 ± 0.005 N.S	0.038 ± 0.004 N.S

Data are expressed as mean ± SE.  
T1, T2, T3 = Treated group with Patulin  
\*\* = Highly significant ( $P \leq 0.01$ )  
N.S = Non significant ( $P > 0.05$ )

Number of samples in each group is 10.  
Significant change in comparison between groups:  
\* = Significant ( $0.01 < P \leq 0.05$ )

The level of AST remained elevated in two weeks (T2) group compared to that of ALT level.

From the previous data a highly significant decline of AST level was found in three weeks (T3) group as

compared to two weeks (T2) group, but the level of ALT showed highly significant decrease in two weeks (T2) group compared with one week (T1) group which means gradual recovery of hepatocytes. This means that falling aminotransferase suggests a decrease in hepatocellular damage (Marshall and Bangert, 2004) which was proved in this study by improvement of histological changes in hepatocytes. However, the decrease in Patulin hepatotoxicity in appeared in (T2) and (T3) groups could be explained either by the possibility of metabolic conversion of Patulin into less cytotoxic compound or due to the adaptation of hepatocytes via detoxification process. Another explanation based on its excretion via urine or feces (Moss, 2002; Speijers, 2004).

Otherwise, GGT is a key enzyme implicated in the homeostasis of intracellular reduced glutathione (GSH) and hence in the regulation of the cellular redox state. Besides, the extracellular cleavage of GSH by GGT leads to reactive oxygen species (ROS) production (Accaoui et al., 2000). Moreover, it is known that oxidative conditions occurring during inflammation that evoked by tissue damage can induce the expression of GGT (Zhang et al., 2006).

The results of TNF- $\alpha$  concentration in serum rats summarized in table (3), pointed that the concentration was gradually increased between groups in manner related to the duration of treatment. These results are in agreement with those obtained by AL-Anati et al. (2005). TNF- $\alpha$  was reported to induce both necrosis and apoptosis of hepatocytes and the increased concentrations of TNF- $\alpha$  in serum indicated that the production of this cytokine may also be induced (Decicco et al., 1998). These findings indicated that several mycotoxins are able to modulate the production of cytokines in different organs and cell types (Bondy and Pestka, 2000; Oswald and Come'ra, 1998) which signified that Patulin treatment results in the ROS production in mammalian cells and ROS partially contributes to PAT- induced cytotoxicity (Liu et al., 2007).

Ferritin is used for assessment of malignant disease and liver disorders (Sirus and Yusuf, 2006). It is a major iron-storage molecule in the human body cells and has thus been used for decades as a diagnostic indicator of iron concentration in liver and other tissues (Finch et al., 1986; Hasan et al., 2006). Intracellular iron can participate in the formation of free radicals, leading to liver cell damage. This may be prevented by the ability of ferritin to oxidize and store iron (Hagen et al., 2002). However, in liver diseases, redox is increased thereby damaging the hepatic tissue (Muriel, 2009).

The data in the present work, table (3) revealed that Patulin induced highly significant decrease in the mean value of ferritin concentration

in three weeks group (T3) only. While a non significant decrease in the mean value of ferritin concentrations were noticed after one and two weeks groups (T1, T2) which indicate that the interaction of free radicals, as a result of Patulin accumulation in (T3) group. Ferritin is probably also involved in the pathogenesis of some inflammatory diseases including hepatitis (Biemond et al., 1988). The decrease of ferritin level may be attributed to the induction of oxidative stress by Patulin (Liu et al., 2007) leading to the disruption in iron homeostasis (Erikson et al., 2006) which resulting from impaired liver function (Ganz, 2009).

Concentrations of ferritin in serum increase in a variety of conditions that include cancer, liver disease, inflammation, iron overload, or iron treatment (Tran et al., 1997). Hann et al. (2006) supported that increased serum ferritin levels are associated with an increased risk of primary hepatocellular carcinoma (PHC) which contradict with this work results.

Carbohydrates serve numerous functions in the body, but the bulk of the carbohydrate ingested is used to derive energy for metabolism. The level of glucose in blood of normal animals, and consequently the amounts available to body organs, is closely regulated through the action of several hormones. When carbohydrate intake is high, excess glucose is converted to a glycogen, a glucose polymer, for storage, but the amount of glycogen storage is limited (Donaldson, 2001).

Our results in table (3) revealed that the mean value of glucose concentration was not changed in Patulin treated group for one week (T1) but highly significant increased in treated groups for two (T2) and three (T3) weeks compared to the control group. These results were confirmed by Devaraj et al. (1983) in which Patulin is found to interfere with the carbohydrate metabolism. Otherwise, Sakithisekaran et al. (1989) and Wouters and Speijers (1996) demonstrated that via the gluconeogenesis which is stimulated by increase glucose-6-phosphatase and fructose-1, 6-diphosphatase activity. Patulin was proved to be diabetogenic by Devaraj et al. (1986) who explained the elevation of fasting blood glucose level through glucose tolerance test which revealed an elevated glucose curve and reduced insulin production. Also, Speijers (2004) reported that Patulin increase blood glucose level by 60 %. By looking to table (3), the mean value of liver glycogen concentration was slightly decreased but not significant in treated groups compared to control group. These results were in accord with the study by Suzuki et al (1974) who found depletion of glycogen in liver after citrinin toxicity. Also, Rastogi et al

(2000) reported that single doses of aflatoxin B1 decreased liver glycogen.

In addition, the present work was done to examine the protecting effect of crude venom extracted from jelly fish *Cassiopea Andromeda* against Patulin hepatotoxicity through bradykinnin content that was the effector peptide of kallikrein-kinin system. From the obtained data, table (4) the serum level of AST, ALT and GGT enzymes showed highly significant decrease in (T4) group as compared to (T1) group and as previously reported in table (3), these transaminases and GGT were highly significant increased in (T1) group compared to control group. Crude venom extracted from jelly fish, *Eutonina indicans* was proved by Abdel-Rehim et al. (1996) to have no effect on liver enzymes activities which indicate its safty use in lab animals. So,

decreased aminotransferases was indicator to reduced liver damage (Sanchro-Bru et al., 2007). This means that extracted crude venom of jelly fish *Cassiopea Andromeda* ameliorate the hepatotoxic effect of Patulin administrated for one week.

On the other hand, only the level of AST was still highly significant increase in (T4) group compared with control group. This result was coincident with the studies done by Omran and Abdel-Rahman (1992); Assi and Nasser (1999); Pipelzadeh et al. (2006) who using crude venoms of different sources. This increase may due to the presence of AST in organs other than the liver. Thus AST level in rats has a limited value as specific endpoint in toxicological studies (Bondy et al., 2000).

**Table 4:** Effect of Crude Venom (1.78 mg/ 20 g b.w) on Some Biochemical Parameters in Male Rats after Patulin (0.2mg/kg b.w.) Hepatotoxicity.

Parameter	Control	T 1	T 4
AST U/L	35.146 ± 0.42	70.656±0.79	51.253±0.71
			**
		●●	●●
ALT U/L	41.073 ± 0.68	45.341 ± 0.97	41.232 ± 0.84
			**
		●●	N.S
GGT U/L	8.203 ± 0.21	9.834 ± 0.41	8.819 ± 0.24
			*
		●●	N.S
TNF Pg/ml	38.027 ± 0.97	53.925 ± 0.81	46.445 ± 0.67
			**
		●●	●●
Ferritin µg/ml	1.617 ± 0.03	1.605 ± 0.06	1.607 ± 0.03
			N.S
		N.S	N.S
Glucose mmol/l	3.495 ± 0.11	3.356 ± 0.14	3.217 ± 0.08
			N.S
		N.S	N.S
Glycogen µg/µl	0.052 ± 0.01	0.038 ± 0.004	0.037 ± 0.003
			N.S
		N.S	N.S

Data are expressed as mean ± SE. Number of sample in each group is 10.

T1= Treated group with Patulin T4 = Treated group with Patulin + crude venom of jelly fish

Significant change in comparison between groups: \*\*, ●● = Highly significant (P ≤ 0.01)

\* = Significant (0.01 < P ≤ 0.05) N.S = Non significant (P > 0.05)

First significant change as compared with T1,

Second significant change as compared with control.

By the examination of the effect of jelly fish crude venom on Patulin toxicity in rats, the crude venom was found to decrease significantly the concentration of tumor necrosis factor-alpha (TNF-α) in (T4) group compared with (T1) group. This improvement may be attributed to the protective effects of crude venom bradykinnin and related polypeptide (Burnett and Calton, 1977). On the other hand, the bradykinnin was known to stimulate the release of prostaglandins (PGs) in a variety of animal

tissues (Levant et al., 2006). PGs in turn was reported to improve the hepatic function and structure (Lkeya et al., 2002; Fouda, 2004; Rincon-Sanchez et al., 2005) and required for liver regeneration and involved in the regulation of a number of cytokines (Rudnick et al., 2001) acting to limit TNF-α. The mechanisms by which prostaglandins limit TNF-α mRNA levels may underlie endogenous regulatory mechanisms that limit inflammation, and may have important implications for understanding chronic

inflammatory disease pathogenesis (Stafford and Marnett, 2007).

Otherwise, the mean value of TNF- $\alpha$  was highly increased in (T4) group compared to control group. Increased serum concentration of TNF- $\alpha$  as one of the small peptide molecules which act as an important mediator in the regulation of the immune and inflammatory responses (Bouhet and Oswald, 2005) indicated that organs or tissue other than the liver could be affected (Decicco et al., 1998).

The obtained data from the present investigation (table 4) revealed that the mean value of ferritin, glucose and glycogen concentrations were not significantly changed in (T4) group compared to (T1) group and also control group. This phenomenon indicates that the Patulin had no effect on these parameters in (T1) group and parallel to each other on the same results of (T4) group. In the present study, it was observed that administration of Patulin (0.2 mg/kg b.w.) to laboratory rats, affects the histological structure of liver parenchyma. Individual variation regarding sensitivity to toxic effect was observed in different animals and similar observation was reported by El-Sawi and Habib (2004) as a sign of multi drug gene resistance. The effect of Patulin on liver parenchyma varies in different weeks. In animal group of one week (T1), there was evident hepatocytes vacuolation and damage of cellular membrane (necrosis), while in group of two weeks (T2) the histological changes were less compared to those of one week (T1). Instead of vacuolation, scattered apoptotic cells were observed in groups of two weeks (T2) and three weeks (T3). The proliferation of bile ducts in group of three weeks (T3) was described to be associated with hepatic metabolic disturbance or toxic exposure (George et al., 2001). The nuclei showed marked variation in size, Pyknosis (small dark nuclei), karyomegally (large sized) and karyolysis (degeneration) were observed. The enlarged nuclei was observed mainly in animals given Patulin for two weeks (T2) and three weeks (T3) and also in jelly fish treated group (T4) which may as a result of hepatocytes toxicity (Fong et al., 2004).

The significant elevation of liver enzymes observed in the present study in treated groups could be explained in view of histological finding which showed cellular membrane damage especially in group of one week (T1) where the specific liver enzyme ALT was high. Its value was returned to normal level in groups of two weeks (T2) and three weeks (T3). This support histological observation where hepatocytes appeared with intact outlines and only focal apoptosis were observed. The focal damage in hepatocytes of two weeks group (T2) could explain the sustained elevation of AST and

GGT levels in this group. Similar association between biochemical finding and histological changes was reported in literature (Wu et al., 2007).

TNF- $\alpha$  was a known cytokine secreted by many cell types including kupffer cells, and macrophages (Gonzalez-Amaro et al., 1994). In the present study Von Kupffer cells, a well known fixed phagocyte of liver, were found to be increased. TNF- $\alpha$  was reported by Fong et al. (2004) to induces apoptosis in target cells that express TNF- $\alpha$  receptors on their cell membrane. Apoptotic hepatocytes were observed especially in groups of two weeks (T2) and three weeks (T3) where TNF- $\alpha$  was described to be significantly high in their serum. Apoptosis was reported to be an effective process for the elimination of unneeded, diseased or transformed cells (D'agostini et al., 2001). It causes cell death in a way that differs morphologically and biochemically from necrosis. Earlier studies

Apoptosis was reported to be an effective process for the elimination of unneeded, diseased or transformed cells (D'agostini et al., 2001). It causes cell death in a way that differs morphologically and biochemically from necrosis. Earlier studies showed that the common core mechanism of apoptosis is a DNA fragmentation and nuclear morphological lesions, such as condensation and fragmentation (Schwartman and Cidlowski, 1993). In this study apoptotic hepatocytes showed dark stained cytoplasm and condensed small nuclei.

The current study showed scattered apoptotic cells in groups of two weeks (T2) and three weeks (T3) which are in accord with what was reported with other mycotoxins such as ochratoxin A (Bokhari and Ali 2006). Also, Atroshi et al. (2000) reported that any given toxins may induce both apoptosis and necrosis, depending on the dose and period of administration and attributed the changes to the increase in reactive oxygen species (ROS).

On the other hand, inflammation plays a role in classical chemical toxicity as follows: initial toxic injury produces focal tissue damage and necrosis in target organ (Luster et al., 2001). Necrosis and cellular vacuolation were initially observed in group after one week (T1) of Patulin administration. The same authors reported that as a result of cellular damage (necrosis), tissue fixed macrophages, are activated and secrete inflammatory products including the proinflammatory cytokine tumour necrosis factor TNF- $\alpha$ , which is a central regulator that aids in tissue repair by stimulating apoptosis and cell proliferation as well as exacerbating cell damage by initiating an overly aggressive inflammatory process. The same was observed in the next groups of Patulin administration after two weeks (T2) and three weeks (T3) where apoptosis was dominant over

necrosis. The inability of the system to neutralize the excessive release of reactive oxygen species are responsible for cell damage or activating genes responsible for cell proliferation (Luster et al., 2001).

To our knowledge, there is no detailed information regarding Patulin pharmacokinetics in human (absorption and metabolism) or toxicokinetic behaviour. Future studies were needed to investigate the cumulative chronic exposure to such mycotoxin. The question here, can the crude venom of jelly fish in the present work use as a new therapeutic approach for enhancing hepatocytes detoxification of mycotoxins? The present work recommended that different feedstuffs or fruit juices known to be liable for mycotoxin contamination must be carried out and checked periodically before popular consuming.

#### 4. Conclusions

The present study indicated that the biochemical and histological effect of Patulin was time dependent. Variation in degree of response was observed in different animals in the same group. Also, the results were pointed to the potential protective effect of crude venom extracted from jelly fish *Cassiopea Andromeda* which can be useful to protect humans or animals against the adverse health effects of this mycotoxin. This study could be considered preliminary as there are no published studies dealing with Patulin hepatotoxicity on studied parameters.

#### Acknowledgments

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## New exact solutions for the Zhiber-Shabat equation using the extended F-expansion method

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**Abstract:** Extended F-expansion method is proposed to seek exact solutions of the Zhiber-Shabat (ZS) equation. As a result, many new and more general exact solutions are obtained. Interesting Jacobi doubly periodic wave solutions is obtained from the F-expansion (EFE) method with symbolic computation. It is shown that soliton solutions and triangular periodic solutions can be established as the limits of Jacobi doubly periodic wave solutions. In addition, as an illustrative sample, the properties for the Jacobi doubly periodic wave solutions of these equations are shown with some figures.

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**Keywords:** Extended F-expansion method; Exact solutions; Liouville equation; Dodd-Bullough-Mikhailov equation; Tzitzeica-Dodd-Bullough equation; sinh-Gordon equation; Zhiber-Shabat equation.

### 1. Introduction

Conte and Musette [1], studied Zhiber-Shabat (ZS) equation and obtained two kinds of solutions. Wazwaz [2] obtained six exact solutions of ZS equation by using tanh and extended tanh methods. Tang et al. [3] considered the existence of bounded travelling wave solutions of ZS equation and obtained several travelling wave solutions. Recently, Bin et al. [4] studied ZS equation using the bifurcation theory and the method of phase portraits analysis and obtained many solitary wave solutions, compacton solutions and smooth periodic wave

solutions. Moreover, the  $\frac{G'}{G}$ -expansion method is introduced to solve ZS equation by Borhanifar and Moghanlu [5].

In this paper, we will apply the extended F-expansion method to study ZS equation

$$u_{xt} + \beta e^u + \gamma e^{-u} + \lambda e^{-2u} = 0, \quad (1)$$

where  $\beta$ ,  $\gamma$  and  $\lambda$  are arbitrary constants. If we take  $\gamma = \lambda = 0$ , Eq. (1) reduced to the Liouville equation [6]. If we take  $\lambda = 0$ , Eq. (1) reduced to the sinh-Gordon equation [7]. And for  $\gamma = 0$ , (1) reduced to the well-known Dodd-Bullough-Mikhailov equation [8]. Moreover, for  $\beta = 0$ ,  $\gamma = -1$ ,  $\lambda = 1$ , (1) reduced to the Tzitzeica-Dodd-Bullough equation [8]. The previous equations plays an important role in many scientific applications such as solid state physics, nonlinear optics, dusty plasma, plasma physics, fluid dynamics, mathematical biology, nonlinear optics, dislocations in crystals, kink dynamics, and chemical kinetics, and quantum

field theory. Moreover many authors have studied these equation (see for instance, [9]-[10]).

It is known that many physical phenomena are often described by nonlinear evolution equations (NLEEs). Integrable systems and NLEEs have recently attracted much attention of mathematicians as well as physicists. Many methods for obtaining explicit travelling solitary wave solutions to NLEEs have been proposed. Among these are the tanh methods [11]-[12],  $\frac{G'}{G}$ -expansion method [13]-[14],

the exp-function method [15]-[16], Jacobi and extended Jacobi elliptic function expansion methods [17]-[18], the inverse scattering transform [19]- [20] and so on. Recently F-expansion method [21]-[25] was proposed to obtain periodic wave solutions of NLEEs, which can be thought of as a concentration of JEF expansion since F here stands for everyone of JEFs.

In this paper, we apply the extended F-expansion (EFE) method with symbolic computation to Eq. (1) for constructing their interesting Jacobi doubly periodic wave solutions. It is shown that soliton solutions and triangular periodic solutions can be established as the limits of Jacobi doubly periodic wave solutions. In addition the algorithm that we use here also a computerized method, in which generating an algebraic system.

### 2. Material and Methods

In this section, we introduce a simple description of the EFE method, for a given partial differential equation .

$$G(u, u_x, u_t, u_{xt}, \dots) = 0. \quad (2)$$

We like to know whether travelling waves (or stationary waves) are solutions of Eq. (2). The first step is to unite the independent variables  $x$  and  $t$  into one particular variable through the new variable

$$\zeta = x - vt, \quad u(x, t) = U(\zeta),$$

where  $V$  is wave speed, and reduce Eq. (2) to an ordinary differential equation(ODE)

$$G(U, U', U'', U''', \dots) = 0. \tag{3}$$

Our main goal is to derive exact or at least approximate solutions, if possible, for this ODE. For this purpose, let us simply  $U$  as the expansion in the form,

$$u(x, t) = U(\zeta) = \sum_{i=0}^N a_i F^i + \sum_{i=1}^N a_{-i} F^{-i}, \tag{4}$$

where

$$F' = \sqrt{A + BF^2 + CF^4}, \tag{5}$$

the highest degree of  $\frac{d^p U}{d\zeta^p}$  is taken as

$$O\left(\frac{d^p U}{d\zeta^p}\right) = N + p, \quad p = 1, 2, 3, \dots, \tag{6}$$

$$O\left(U^q \frac{d^p U}{d\zeta^p}\right) = (q+1)N + p, \quad q = 0, 1, 2, \dots, p = 1, 2, 3, \dots. \tag{7}$$

Where  $A, B$  and  $C$  are constants, and  $N$  in Eq. (3) is a positive integer that can be determined by balancing the nonlinear term(s) and the highest order derivatives. Normally  $N$  is a positive integer, so that an analytic solution in closed form may be obtained. Substituting Eqs. (2)- (5) into Eq. (3) and comparing the coefficients of each power of  $F(\zeta)$  in both sides, to get an over-determined system of nonlinear algebraic equations with respect to  $v, a_0, a_1, \dots$ . Solving the over-determined system of nonlinear algebraic equations by use of Mathematica. The relations between values of  $A, B, C$  and corresponding JEF solution  $F(\zeta)$  of Eq. (4) are given in Table 1. Substitute the values of  $A, B, C$  and the corresponding JEF solution  $F(\zeta)$  chosen from table 1 into the general form of solution, then an ideal periodic wave solution expressed by JEF can be obtained.

**Table 1:** Relation between values of  $(A, B, C)$  and corresponding  $F$

$A$	$B$	$C$	$F(\zeta)$
1	$-1 - m^2$	$m^2$	$\text{sn}(\zeta),$ $\text{cd}(\zeta) = \frac{\text{cn}(\zeta)}{\text{dn}(\zeta)}$
$1 - m^2$	$2m^2 - 1$	$-m^2$	$\text{cn}(\zeta)$
$m^2 - 1$	$2 - m^2$	$-1$	$\text{dn}(\zeta)$
$m^2$	$-1 - m^2$	1	$\text{ns}(\zeta) = \frac{1}{\text{sn}(\zeta)},$ $\text{dc}(\zeta) = \frac{\text{dn}(\zeta)}{\text{cn}(\zeta)}$
$-m^2$	$2m^2 - 1$	$1 - m^2$	$\text{nc}(\zeta) = \frac{1}{\text{cn}(\zeta)}$
$-1$	$2 - m^2$	$m^2 - 1$	$\text{nd}(\zeta) = \frac{1}{\text{dn}(\zeta)}$
1	$2 - m^2$	$1 - m^2$	$\text{sc}(\zeta) = \frac{\text{sn}(\zeta)}{\text{cn}(\zeta)}$
1	$2m^2 - 1$	$-m^2 (-1 - m^2)$	$\text{sd}(\zeta) = \frac{\text{sn}(\zeta)}{\text{dn}(\zeta)}$
$1 - m^2$	$2 - m^2$	1	$\text{cs}(\zeta) = \frac{\text{cn}(\zeta)}{\text{sn}(\zeta)}$
$-m^2 (1 - m^2)$	$2m^2 - 1$	1	$\text{ds}(\zeta) = \frac{\text{dn}(\zeta)}{\text{sn}(\zeta)}$
$\frac{1}{4}$	$\frac{1 - 2m^2}{2}$	$\frac{1}{4}$	$\text{ns}(\zeta) + \text{cs}(\zeta)$
$\frac{1 - m^2}{4}$	$\frac{1 + m^2}{2}$	$\frac{1 - m^2}{2}$	$\text{nc}(\zeta) + \text{sc}(\zeta)$
$\frac{1}{4}$	$\frac{m^2 - 2}{2}$	$\frac{m^2}{4}$	$\text{ns}(\zeta) + \text{ds}(\zeta)$
$\frac{m^2}{4}$	$\frac{m^2 - 2}{2}$	$\frac{m^2}{4}$	$\text{sn}(\zeta) + \text{ics}(\zeta)$

where  $\text{sn}(\zeta)$ ,  $\text{cn}(\zeta)$  and  $\text{dn}(\zeta)$  are the JE sine function, JE cosine function and the JEF of the third kind, respectively. And

$$\text{cn}^2(\zeta) = 1 - \text{sn}^2(\zeta), \text{dn}^2(\zeta) = 1 - m^2 \text{sn}^2(\zeta), \quad (8)$$

with the modulus  $m$  ( $0 < m < 1$ ).

When  $m \rightarrow 1$ , the Jacobi functions degenerate to the hyperbolic functions, i.e.,

$$\text{sn}\zeta \rightarrow \tanh\zeta, \quad \text{cn}\zeta \rightarrow \text{sech}\zeta, \quad \text{dn}\zeta \rightarrow \text{sech}\zeta,$$

when  $m \rightarrow 0$ , the Jacobi functions degenerate to the triangular functions, i.e.,

$$\text{sn}\zeta \rightarrow \sin\zeta, \quad \text{cn}\zeta \rightarrow \cos\zeta \quad \text{and} \quad \text{dn} \rightarrow 1.$$

### 3. Results

In this section, we will apply the extended method to study ZS equation (1)

$$u_{,xt} + \beta e^u + \gamma e^{-u} + \lambda e^{-2u} = 0, \quad (9)$$

if we use  $\zeta = x - vt$ ,  $u(x, t) = U(\zeta)$  carries Eq. (9) into an ODE

$$-vU'' + \beta e^U + \gamma e^{-U} + \lambda e^{-2U} = 0, \quad (10)$$

if we use  $V = e^U$  carries Eq. (10) into:-

$$-v(VV'' - (V')^2) + \beta V^3 + \gamma V + \lambda = 0. \quad (11)$$

Balancing the term  $VV''$  with the term  $V^3$  we obtain  $N = 2$  then

$$\begin{aligned} V(\zeta) &= a_0 + a_1 F + a_{-1} F^{-1} + a_2 F^2 + a_{-2} F^{-2}, \\ F' &= \sqrt{A + BF^2 + CF^4}. \end{aligned} \quad (12)$$

Substituting Eq. (12) into Eq. (11) and comparing the coefficients of each power of  $F$  in both sides, to get an over-determined system of nonlinear algebraic equations with respect to  $v$ ,  $a_i$ ,  $i = 1, -1, 2, -2$ . Solving the over-determined system of nonlinear algebraic equations by use of Mathematica, we obtain three groups of constants:

1.

$$\begin{aligned} a_1 &= a_{-1} = 0, \quad a_0 = \frac{2Bv - \sqrt{4(B^2 + 12AC)v^2 - 3\beta\gamma}}{3\beta}, \\ a_2 &= \frac{2Cv}{\beta}, \quad a_{-2} = \frac{2Av}{\beta}, \\ \lambda &= \frac{1}{27\beta^2} (2(3\beta\gamma + 8B^2v^2 + 96ACv^2)\sqrt{4(B^2 + 12AC)v^2 - 3\beta\gamma} \\ &\quad - 16B^3v^3 + 576ABCv^3), \end{aligned} \quad (13)$$

2.

$$\begin{aligned} a_1 &= a_{-1} = a^2 = 0, \\ a_0 &= \frac{2Bv - \sqrt{4(B^2 - 3AC)v^2 - 3\beta\gamma}}{3\beta}, \quad a_{-2} = \frac{2Av}{\beta}, \\ \lambda &= \frac{1}{27\beta^2} (2(3\beta\gamma + 8B^2v^2 + 96ACv^2)\sqrt{4(B^2 - 3AC)v^2 - 3\beta\gamma} \\ &\quad - 16B^3v^3 + 72ABCv^3), \end{aligned} \quad (14)$$

3.

$$\begin{aligned} a_1 &= a_{-1} = a^{-2} = 0, \\ a_0 &= \frac{2Bv - \sqrt{4(B^2 - 3AC)v^2 - 3\beta\gamma}}{3\beta}, \quad a_2 = \frac{2Cv}{\beta}, \\ \lambda &= \frac{1}{27\beta^2} (2(3\beta\gamma + 8B^2v^2 + 96ACv^2)\sqrt{4(B^2 - 3AC)v^2 - 3\beta\gamma} \\ &\quad - 16B^3v^3 + 72ABCv^3). \end{aligned} \quad (15)$$

Now, the solutions of ZS equation (9) can be written as follows:

$$\begin{aligned} u_1 &= \ln\left[-\frac{2(1+m^2)v + \sqrt{4(12m^2 + (1+m^2)^2)v^2 - 3\beta\gamma}}{3\beta}\right. \\ &\quad \left. + \frac{2m^2v}{\beta} \text{sn}^2(x-vt) + \frac{2v}{\beta} \text{ns}^2(x-vt)\right], \end{aligned} \quad (16)$$

$$\begin{aligned} u_2 &= \ln\left[-\frac{2(1+m^2)v + \sqrt{4(12m^2 + (1+m^2)^2)v^2 - 3\beta\gamma}}{3\beta}\right. \\ &\quad \left. + \frac{2m^2v}{\beta} \text{cd}^2(x-vt) + \frac{2v}{\beta} \text{dc}^2(x-vt)\right], \end{aligned} \quad (17)$$

$$\begin{aligned} u_3 &= \ln\left[-\frac{2(1-2m^2)v + \sqrt{4(12m^2(m^2-1) + (m^2-1)^2)v^2 - 3\beta\gamma}}{3\beta}\right. \\ &\quad \left. - \frac{2m^2v}{\beta} \text{cn}^2(x-vt) + \frac{2(1-m^2)v}{\beta} \text{nc}^2(x-vt)\right], \end{aligned} \quad (18)$$

$$\begin{aligned} u_4 &= \ln\left[-\frac{2(m^2-2)v + \sqrt{4((2-m^2)^2 + 12(1-m^2)v^2 - 3\beta\gamma}}{3\beta}\right. \\ &\quad \left. - \frac{2v}{\beta} \text{dn}^2(x-vt) + \frac{2(m^2-1)v}{\beta} \text{nd}^2(x-vt)\right], \end{aligned} \quad (19)$$

$$u_5 = \ln\left[-\frac{2(m^2-2)v + \sqrt{4(12(1-m^2) + (2-m^2)^2)v^2 - 3\beta\gamma}}{3\beta} + \frac{2v}{\beta}cs^2(x-vt) + \frac{2v(1-m^2)}{\beta}sc^2(x-vt)\right], \quad (20)$$

$$u_6 = \ln\left[-\frac{2(1-2m^2)v + \sqrt{4(12m^2(1+m^2) + (1-2m^2)^2)v^2 - 3\beta\gamma}}{3\beta} + \frac{2v}{\beta}ds^2(x-vt) + \frac{2vm^2(1+m^2)}{\beta}sd^2(x-vt)\right], \quad (21)$$

$$u_7 = \ln\left[-\frac{2(m^2-0.5)v + \sqrt{4(0.75 + (0.5-m^2)^2)v^2 - 3\beta\gamma}}{3\beta} + \frac{v}{2\beta}(ns(x-vt) + cs(x-vt))^2 + \frac{v}{2\beta}(ns(x-vt) + cs(x-vt))^{-2}\right], \quad (22)$$

$$u_8 = \ln\left[\frac{1}{3\beta}(2(0.5+0.5m^2)v - \sqrt{4(12(0.5-0.5m^2)(0.25-0.25m^2) + (0.5+0.5m^2)^2)v^2 - 3\beta\gamma}) + \frac{2v(0.5-0.5m^2)}{\beta}(nc(x-vt) + sc(x-vt))^2 + \frac{2v(0.5-0.5m^2)}{\beta}(nc(x-vt) + sc(x-vt))^{-2}\right], \quad (23)$$

$$u_9 = \ln\left[-\frac{2(0.5m^2-1)v + \sqrt{4(0.75m^2 + (1-0.5m^2)^2)v^2 - 3\beta\gamma}}{3\beta} + \frac{m^2v}{2\beta}(ns(x-vt) + ds(x-vt))^2 + \frac{v}{2\beta}(ns(x-vt) + ds(x-vt))^{-2}\right], \quad (24)$$

$$u_{10} = \ln\left[-\frac{2(1-0.5m^2)v + \sqrt{4(0.75m^4 + (1-0.5m^2)^2)v^2 - 3\beta\gamma}}{3\beta} + \frac{m^2v}{2\beta}(sn(x-vt) + ics(x-vt))^2 + \frac{m^2v}{2\beta}(sn(x-vt) + ics(x-vt))^{-2}\right], \quad (25)$$

$$u_{11} = \ln\left[-\frac{2(1+m^2)v + \sqrt{4((1+m^2)^2 - 3m^2)v^2 - 3\beta\gamma}}{3\beta} + \frac{2v}{\beta}ns^2(x-vt)\right], \quad (26)$$

$$u_{12} = \ln\left[-\frac{2(1+m^2)v + \sqrt{4((1+m^2)^2 - 3m^2)v^2 - 3\beta\gamma}}{3\beta} + \frac{2v}{\beta}dc^2(x-vt)\right], \quad (27)$$

$$u_{13} = \ln\left[-\frac{2(1-2m^2)v + \sqrt{4(3m^2(1-m^2) + (2m^2-1)^2)v^2 - 3\beta\gamma}}{3\beta} + \frac{2(1-m^2)v}{\beta}nc^2(x-vt)\right], \quad (28)$$

$$u_{14} = \ln\left[-\frac{2(m^2-2)v + \sqrt{4((2-m^2)^2 - 3(1-m^2))v^2 - 3\beta\gamma}}{3\beta} + \frac{2(m^2-1)v}{\beta}nd^2(x-vt)\right], \quad (29)$$

$$u_{15} = \ln\left[-\frac{2(m^2-2)v + \sqrt{4(3(m^2-1) + (2-m^2)^2)v^2 - 3\beta\gamma}}{3\beta} + \frac{2v(1-m^2)}{\beta}sc^2(x-vt)\right], \quad (30)$$

$$u_{16} = \ln\left[-\frac{2(1-2m^2)v + \sqrt{4((1-2m^2)^2 - 3m^2(1+m^2))v^2 - 3\beta\gamma}}{3\beta} + \frac{2vm^2(1+m^2)}{\beta}sd^2(x-vt)\right], \quad (31)$$

$$u_{17} = \ln\left[-\frac{2(m^2-0.5)v + \sqrt{4((0.5-m^2)^2 - \frac{3}{16})v^2 - 3\beta\gamma}}{3\beta} + \frac{v}{2\beta}(ns(x-vt) + cs(x-vt))^{-2}\right], \quad (32)$$

$$u_{18} = \ln\left[\frac{1}{3\beta}(2(0.5+0.5m^2)v - \sqrt{4(3(0.5m^2-0.5)(0.25-0.25m^2) + (0.5+0.5m^2)^2)v^2 - 3\beta\gamma}) + \frac{2v(0.5-0.5m^2)}{\beta}(nc(x-vt) + sc(x-vt))^{-2}\right], \quad (33)$$

$$u_{19} = \ln\left[-\frac{2(0.5m^2 - 1)v + \sqrt{4((1 - 0.5m^2)^2 - \frac{3m^2}{16})v^2 - 3\beta\gamma}}{3\beta} + \frac{v}{2\beta}(ns(x - vt) + ds(x - vt))^{-2}\right], \quad (34)$$

$$u_{20} = \ln\left[-\frac{2(1 - 0.5m^2)v + \sqrt{4((1 - 0.5m^2)^2 - \frac{3m^4}{16})v^2 - 3\beta\gamma}}{3\beta} + \frac{m^2v}{2\beta}(sn(x - vt) + ics(x - vt))^{-2}\right], \quad (35)$$

$$u_{21} = \ln\left[-\frac{2(1 + m^2)v + \sqrt{4((1 + m^2)^2 - 3m^2)v^2 - 3\beta\gamma}}{3\beta} + \frac{2m^2v}{\beta}sn^2(x - vt)\right], \quad (36)$$

$$u_{22} = \ln\left[-\frac{2(1 + m^2)v + \sqrt{4((1 + m^2)^2 - 3m^2)v^2 - 3\beta\gamma}}{3\beta} + \frac{2m^2v}{\beta}cd^2(x - vt)\right], \quad (37)$$

$$u_{23} = \ln\left[-\frac{2(1 - 2m^2)v + \sqrt{4(3m^2(1 - m^2) + (2m^2 - 1)^2v^2) - 3\beta\gamma}}{3\beta} - \frac{2m^2v}{\beta}cn^2(x - vt)\right], \quad (38)$$

$$u_{24} = \ln\left[-\frac{2(m^2 - 2)v + \sqrt{4((2 - m^2)^2 - 3(1 - m^2))v^2 - 3\beta\gamma}}{3\beta} - \frac{2v}{\beta}dn^2(x - vt)\right], \quad (39)$$

$$u_{25} = \ln\left[-\frac{2(m^2 - 2)v + \sqrt{4(3(m^2 - 1) + (2 - m^2)^2)v^2 - 3\beta\gamma}}{3\beta} + \frac{2v}{\beta}cs^2(x - vt)\right], \quad (40)$$

$$u_{26} = \ln\left[-\frac{2(1 - 2m^2)v + \sqrt{4((1 - 2m^2)^2 - 3m^2(1 + m^2))v^2 - 3\beta\gamma}}{3\beta} + \frac{2v}{\beta}ds^2(x - vt)\right], \quad (41)$$

$$u_{27} = \ln\left[-\frac{2(m^2 - 0.5)v + \sqrt{4((0.5 - m^2)^2 - \frac{3}{16})v^2 - 3\beta\gamma}}{3\beta} + \frac{v}{2\beta}(ns(x - vt) + cs(x - vt))^2\right], \quad (42)$$

$$u_{28} = \ln\left[\frac{1}{3\beta}(2(0.5 + 0.5m^2)v - \sqrt{4(3(0.5m^2 - 0.5)(0.25 - 0.25m^2) + (0.5 + 0.5m^2)v^2 - 3\beta\gamma)} + \frac{2v(0.5 - 0.5m^2)}{\beta}(nc(x - vt) + sc(x - vt))^2\right], \quad (43)$$

$$u_{29} = \ln\left[-\frac{2(0.5m^2 - 1)v + \sqrt{4((1 - 0.5m^2)^2 - \frac{3m^2}{16})v^2 - 3\beta\gamma}}{3\beta} + \frac{m^2v}{2\beta}(ns(x - vt) + ds(x - vt))^2\right], \quad (44)$$

$$u_{30} = \ln\left[-\frac{2(1 - 0.5m^2)v + \sqrt{4((1 - 0.5m^2)^2 - \frac{3m^4}{16})v^2 - 3\beta\gamma}}{3\beta} + \frac{m^2v}{2\beta}(sn(x - vt) + ics(x - vt))^2\right]. \quad (45)$$

The modulus of solitary wave solutions  $u_1$ ,  $u_2$ ,  $u_{16}$  and  $u_{22}$  are displayed in figures 1, 2, 3 and 4 respectively, with values of parameters listed in their captions.

Fig. 1 (a)

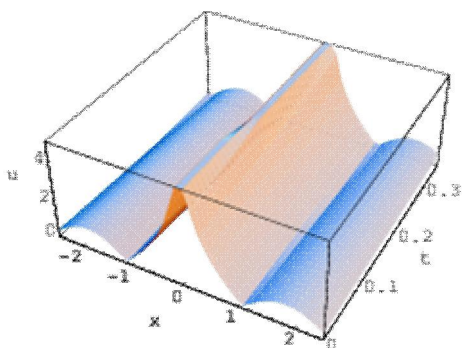


Fig. 1 (b)

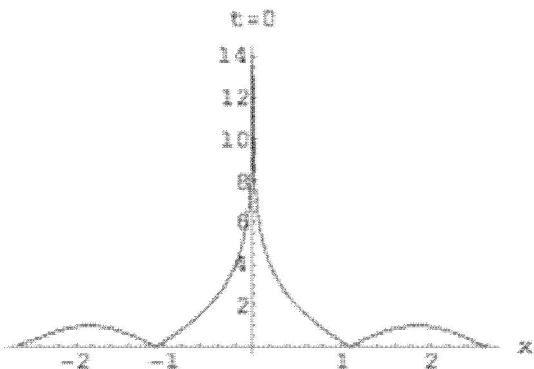


Fig. 1 The modulus of solitary wave solution  $u_1$  (Eq. 16) where  $m = \beta = \gamma = 0.5$ .

Fig. 2 (a)

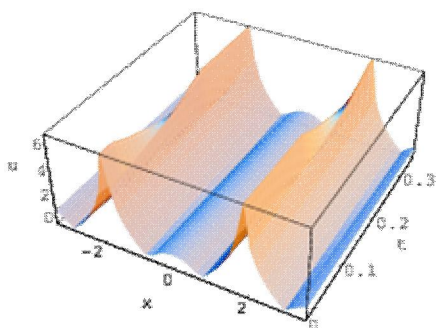


Fig. 2 (b)

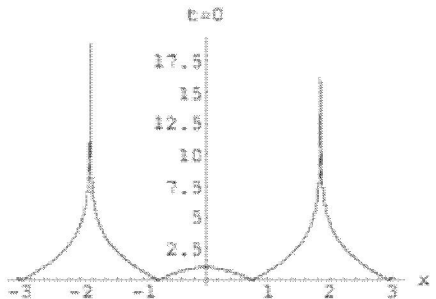


Fig. 2 The modulus of solitary wave solution  $u_2$  (Eq. 17) where  $m = \beta = \gamma = 0.5$ .

Fig. 3 (a)

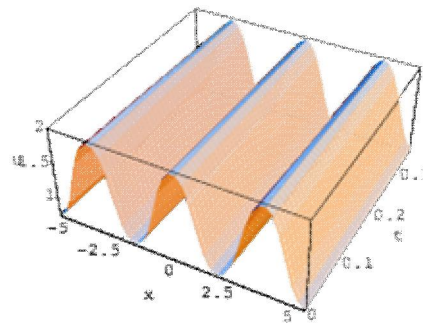


Fig. 3 (b)

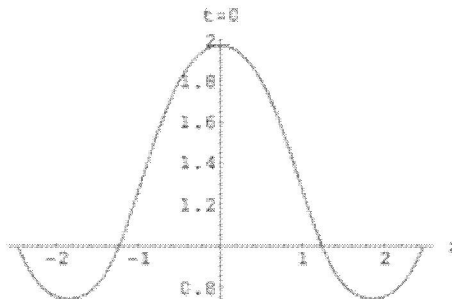


Fig. 3 The modulus of solitary wave solution  $u_{16}$  (Eq. 31) where  $m = \beta = \gamma = 0.5$ .

Fig. 4 (a)

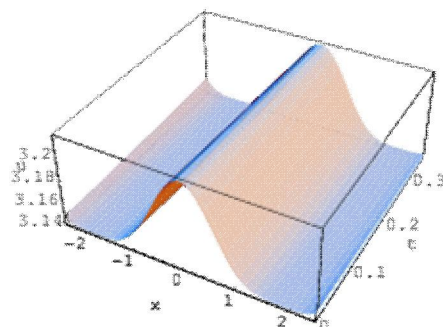


Fig. 4 (b)

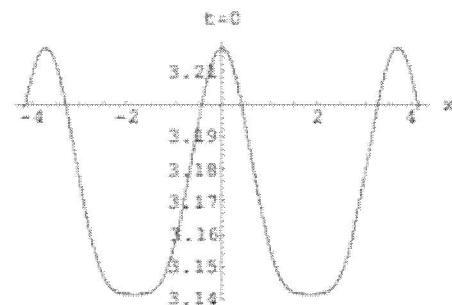


Fig. 4 The modulus of solitary wave solution  $u_{22}$  (Eq. 37) where  $m = \beta = \gamma = 0.5$ .



### 3.1 Soliton solutions

Some solitary wave solutions can be obtained, if the modulus  $m$  approaches to 1 in Eqs. (16)-(43)

$$u_{31} = \ln\left[\frac{4v + \sqrt{4v^2 - 3\beta\gamma}}{3\beta} + \frac{2v}{\beta} \tanh^2(x - vt)\right], \quad (46)$$

$$u_{32} = \ln\left[\frac{4v + \sqrt{4v^2 - 3\beta\gamma}}{3\beta} + \frac{2v}{\beta} \coth^2(x - vt)\right], \quad (47)$$

$$u_{33} = \ln\left[\frac{2v - \sqrt{4v^2 - 3\beta\gamma}}{3\beta} - \frac{2v}{\beta} \operatorname{sech}^2(x - vt)\right], \quad (48)$$

$$u_{34} = \ln\left[\frac{2v - \sqrt{4v^2 - 3\beta\gamma}}{3\beta} + \frac{2v}{\beta} \operatorname{csch}^2(x - vt)\right], \quad (49)$$

$$u_{35} = \ln\left[\frac{2v - i\sqrt{5v^2 + 3\beta\gamma}}{3\beta} + \frac{4v}{\beta} \sinh^2(x - vt)\right], \quad (50)$$

$$u_{36} = \ln\left[\frac{4v - \sqrt{64v^2 - 3\beta\gamma}}{3\beta} + \frac{2v}{\beta} \tanh^2(x - vt) + \frac{2v}{\beta} \coth^2(x - vt)\right], \quad (51)$$

$$u_{37} = \ln\left[\frac{-v - \sqrt{\frac{v^2}{4} - 3\beta\gamma}}{3\beta} + \frac{v}{2\beta} (\tanh(x - vt) + \operatorname{icsch}(x - vt))^2\right], \quad (52)$$

$$u_{38} = \ln\left[\frac{v - \sqrt{\frac{v^2}{4} - 3\beta\gamma}}{3\beta} + \frac{v}{2\beta} (\coth(x - vt) + \operatorname{csch}(x - vt))^2\right], \quad (53)$$

$$u_{39} = \ln\left[\frac{-v - \sqrt{\frac{v^2}{4} - 3\beta\gamma}}{3\beta} + \frac{v}{2\beta} (\tanh(x - vt) + \operatorname{icsch}(x - vt))^{-2}\right], \quad (54)$$

$$u_{40} = \ln\left[\frac{v - \sqrt{\frac{v^2}{4} - 3\beta\gamma}}{3\beta} + \frac{v}{2\beta} (\coth(x - vt) + \operatorname{csch}(x - vt))^{-2}\right], \quad (55)$$

$$u_{41} = \ln\left[\frac{2v - \sqrt{100v^2 - 3\beta\gamma}}{3\beta} + \frac{2v}{\beta} \operatorname{csch}^2(x - vt) + \frac{4v}{\beta} \sinh^2(x - vt)\right], \quad (56)$$

$$u_{42} = \ln\left[-\frac{v + \sqrt{4v^2 - 3\beta\gamma}}{3\beta} + \frac{v}{2\beta} (\coth(x - vt) + \operatorname{csch}(x - vt))^2 + \frac{v}{2\beta} (\coth^2(x - vt) + \operatorname{csch}^2(x - vt))^{-2}\right], \quad (57)$$

$$u_{43} = \ln\left[\frac{-v - \sqrt{\frac{v^2}{4} - 3\beta\gamma}}{3\beta} + \frac{v}{2\beta} (\tanh(x - vt) + \operatorname{icsch}(x - vt))^2 + \frac{v}{2\beta} (\tanh(x - vt) + \operatorname{icsch}(x - vt))^{-2}\right]. \quad (58)$$

### 3.2 Triangular periodic solutions

Some trigonometric function solutions can be obtained, if the modulus  $m$  approaches to zero in Eqs. (16)-(43)

$$u_{44} = \ln\left[\frac{2v - \sqrt{4v^2 - 3\beta\gamma}}{3\beta} + \frac{2v}{\beta} \cot^2(x - vt)\right], \quad (59)$$

$$u_{45} = \ln\left[\frac{-4v - \sqrt{4v^2 - 3\beta\gamma}}{3\beta} + \frac{2v}{\beta} \csc^2(x - vt)\right], \quad (60)$$

$$u_{46} = \ln\left[\frac{2v - \sqrt{4v^2 - 3\beta\gamma}}{3\beta} + \frac{v}{\beta} \sin^2(x - vt)\right], \quad (61)$$

$$u_{47} = \ln\left[\frac{-2\nu - \sqrt{4\nu^2 - 3\beta\gamma}}{3\beta} + \frac{2\nu}{\beta} \csc^2(x - \nu)\right], \quad (62)$$

$$u_{48} = \ln\left[\frac{-2\nu - \sqrt{4\nu^2 - 3\beta\gamma}}{3\beta} + \frac{2\nu}{\beta} \sec^2(x - \nu)\right], \quad (63)$$

$$u_{49} = \ln\left[\frac{4\nu - \sqrt{4\nu^2 - 3\beta\gamma}}{3\beta} + \frac{2\nu}{\beta} \tan^2(x - \nu)\right], \quad (64)$$

$$u_{50} = \ln\left[\frac{2\nu - \sqrt{4\nu^2 - 3\beta\gamma}}{3\beta} + \frac{2\nu}{\beta} (\csc(x - \nu) + \cot(x - \nu))^{-2}\right], \quad (65)$$

$$u_{51} = \ln\left[\frac{\nu - i\sqrt{2\nu^2 + 3\beta\gamma}}{3\beta} + \frac{\nu}{\beta} (\sec(x - \nu) + \tan(x - \nu))^{-2}\right], \quad (66)$$

$$u_{52} = \ln\left[\frac{2\nu - \sqrt{4\nu^2 - 3\beta\gamma}}{3\beta} + \frac{2\nu}{\beta} (\csc(x - \nu) + \cot(x - \nu))^2\right], \quad (67)$$

$$u_{53} = \ln\left[\frac{\nu - i\sqrt{2\nu^2 + 3\beta\gamma}}{3\beta} + \frac{\nu}{\beta} (\sec(x - \nu) + \tan(x - \nu))^2\right], \quad (68)$$

$$u_{54} = \ln\left[\frac{2\nu - \sqrt{4\nu^2 - 3\beta\gamma}}{3\beta} + \frac{2\nu}{\beta} (\csc(x - \nu) + \cot(x - \nu))^2 + \frac{2\nu}{\beta} (\csc(x - \nu) + \cot(x - \nu))^{-2}\right], \quad (69)$$

$$u_{55} = \ln\left[\frac{\nu - i\sqrt{2\nu^2 + 3\beta\gamma}}{3\beta} + \frac{\nu}{\beta} (\sec(x - \nu) + \tan(x - \nu))^2 + \frac{\nu}{\beta} (\sec(x - \nu) + \tan(x - \nu))^{-2}\right]. \quad (70)$$

Note: The solutions  $u_{31}$ ,  $u_{32}$ ,  $u_{36}$ ,  $u_{44}$  and  $u_{49}$  are the same as in Wazwaz [2]. And the solutions of the special equations in [2] can be obtained where the parameters are taken as special values as pointed in introduction.

#### 4. Discussions

By introducing appropriate transformations and using extended F-expansion method, we have been able to obtain in a unified way with the aid of symbolic computation system-mathematica, a series of solutions including single and the combined Jacobi elliptic function. Also, extended F-expansion method is shown that soliton solutions and triangular periodic solutions can be established as the limits of Jacobi doubly periodic wave solutions. When  $m \rightarrow 1$ , the Jacobi functions degenerate to the hyperbolic functions and given the solutions by the extended hyperbolic functions methods. When  $m \rightarrow 0$ , the Jacobi functions degenerate to the triangular functions and given the solutions by extended triangular functions methods.

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**Physical activity and life style among Male Adolescents in Jeddah, Saudi Arabia**

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**Abstract:** The physical activity among adolescents varies greatly around the world according to the habits, cultures and environmental conditions. Therefore, the present study aimed to investigate the different physical activities among adolescent students in areas Jeddah, Saudi Arabia. **Methods:** This cross-sectional study was conducted during fall 2010 including 10 schools from four geographical areas. The participants were 530 male students from secondary-school Their ages ranged between 16-17 years. Measurements included anthropometric measures (weight, height, and waist circumference), physical activity (walking, jogging/running, biking, swimming, self-defense, etc.) using a validated questionnaire. **Results:** Time in minutes spent per week in different types of physical activity by adolescents revealed non-significant difference in walking weekly, stairs use per day, jogging/running, biking and swimming, where as it was significant in minutes walking per time ( $p=0.013$ ) and minutes biking ( $p=0.006$ ). The  $P$ -value for the one-way ANOVA tests (according to school area) for the sum of all moderate-intensity physical activity  $p=0.002$ ; for the sum of all vigorous-intensity physical activity  $p=0.026$ , and for the total physical activity  $p=0.001$ . The  $P$ -value for the independent sample t-tests (according to clusters) for the sum of all moderate-intensity physical activity,  $p=0.000$ ; for the sum of all vigorous-intensity physical activity,  $p=0.000$ , and for the total physical activity,  $p=0.000$ . **Conclusions:** It is concluded that the physical activity among student adolescents differ significantly from geographical area to another in Jeddah and the youth are suffering from inadequate physical activity.

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**Key word:** Adolescents, geographical regions, physical activity

**1. Introduction**

Recently there have some concerns over the change in the diets and health of adolescence. Historically, the focus has been on the provision of sufficient nutrients and energy in relation to current and future needs, but providing dietary balance and encouraging less sedentary lifestyles are now viewed as the main priorities. These issues are worthy of attention because poor eating and physical activity habits in childhood may increase the risk of health problems in later life (Collison, *et al.* 2010).

Regular physical activity and proper dietary habits can maintain and improve the individuals' physical and mental health and well-being. Furthermore, physical activity participation in youth can be an important way to boost energy expenditure and reduces weight gain.

Some investigators recommended that children and adolescents should accumulate at least 60 minutes of moderate to vigorous physical activity (MVPA) per day (Biddle *et al.*, 2004), but many studies revealed that no more than one third of the adolescents seemed to achieve this physical activity recommendations (Currie *et al.*, 2004; Nilsson *et al.*, 2009). Also, a decline in physical activity from childhood to adolescence has previously been shown (Armstrong & Welsman, 2006, De Cocker *et al.*,

2011). In addition, the American Academy of Pediatrics has recommended that children spend no longer than two hours per day on sedentary activities (American Academy of Pediatrics, 2001). Improper feeding habits and physical activity in childhood may increase the risk of health problems in later life (Collison *et al.*, 2010).

Recently, many cities in Saudi Arabia have observed visible lifestyle changes. This is mainly due to rapid growth in major cities, increased use of technology, availability of high-fats and dense-caloric foods, and reduced occupational-work demands (Al-Hazzaa *et al.*, 2011).

Major risk factors of non-infectious diseases are prevalent in Saudi Arabia including hypertension, hypercholesterolemia, inadequate intake of fruit and vegetables, overweight or obesity, physical inactivity and tobacco use. Most of these risks are closely related to improper diet and physical inactivity (WHO, 2010). Therefore, the present study presents the levels of physical activity and lifestyle habits of Saudi male adolescents from Jeddah.

**2. Material and Methods:****Study sample**

A total of 530 adolescent students were invited to participate in this study from different schools in

geographic localities (South, East, West and North) from Jeddah one of the major cities in Saudi Arabia. A random sample with multistage stratified cluster technique was used to select the sample. The final number of sample size included 106, 105, 161 and 158 school students from the four geographic areas (South, East, West and North) of the city of Jeddah, respectively.

The selected participants were free of any physical health problems. The data were collected during fall 2010. The study protocol and procedures were approved by the Deanship of Scientific Research (DSR), King Abdulaziz University.

#### **Anthropometric measurements**

Anthropometric data included body weight and height. Measurements were performed in the morning by a trained researcher. Body weight was measured to the nearest 100 g using calibrated portable scales. Measurements were done with minimal clothing and without shoes. Height was measured to the nearest centimeter using a calibrated measuring bar while the subject was standing without shoes. Body mass index (BMI) was calculated as the ratio of weight in kilograms by the height squared in meters.

#### **Physical activity questionnaires**

The questionnaire that was used for the assessment of physical activity was previously found to be valid (Al-Hazzaa *et al.*, 2011b). The questionnaire collect information on frequency, duration and intensity of many light-, moderate- and vigorous-intensity physical activities during a typical week, covering as transport and household, fitness and sports activities domains. Activities include walking, jogging/running, swimming, cycling, self-defense, weight training, households, as well as many sports activities such as volleyball, badminton, table tennis, basketball and soccer.

Physical activities were classified into light-, moderate and vigorous-intensity activities based on metabolic equivalent (MET) values according to the compendium of physical activity 21 and the compendium of physical activity for youth.22 Moderate-intensity physical activity includes activities such as normal-pace walking, brisk walking, recreational swimming, household activities and moderate-intensity recreational sports such as. Most household activities were given a mean MET

value of 3 (moderate-intensity activity). Vigorous-intensity physical activity and sports (MET value > 6) included such activities as stair-climbing, jogging and running etc. Physical activity levels were classified into three categories based on the total time per week spent in total physical activity, moderate- and vigorous-intensity physical activities.

#### **Data and statistical analysis**

Data were checked and entered into a computer using an SPSS (SPSS, Inc, Chicago, IL) data file. The maximum total time spent on physical activity per week was made 4 hours for each physical activity per day. Data were then analyzed using SPSS, version 15. Descriptive statistics were presented as means, standard deviations (or standard error) and proportions.

A one-way ANOVA was used to test the differences in physical activity variables across different geographic areas (South, North, West, and East). Cluster Analysis is an exploratory tool designed to reveal natural groupings (or clusters) within our data. It can identify different groups based on various demographic and purchasing characteristics.

#### **3. Results:**

This study included 530 samples from schools from different geographical areas in Jeddah as follows: The numbers of students participating in the study from South, East, West and North were 106, 105, 161 and 158, respectively (Table 1). Their ages ranged between 16.84 and 16.91 years with an average of  $16.88 \pm 0.99$  years, without a significant differences ( $p=0.929$ ) between the four geographical areas in Jeddah.

The standard waist/age ranged from 97.41 to 97.55 cm, with an average of  $97.50 \pm 2.83$  cm, without a significant variation ( $p=0.978$ ,  $F=0.067$ ) among students from four areas in Jeddah. The 3<sup>rd</sup> parameter studied was body weight, which ranged from 96.83 to 73.61, with an average  $71.49 \pm 21.03$  kg, without a significant difference between adolescents from different areas. Concerning the height and BMI it averaged  $168.46 \pm 6.54$  cm and  $25.08 \pm 6.79 \text{ kg/m}^2$ , respectively, without significant differences as shown in table (1)

**Table 1.** Anthropometric characteristics of the participants. One way ANOVA tests were used to compare the mean values according to the school area (total n = 530).

	Area	N	Mean	±SD	F	P-value	Significance
Age (Year)	South	106	16.84	1.00	0.151	0.929	NS
	East	105	16.85	0.92			
	West	161	16.90	0.99			
	North	158	16.91	1.05			
	Total	530	16.88	0.99			
Standard waist/age	South	106	97.41	2.89	0.067	0.978	NS
	East	105	97.47	2.72			
	West	161	97.53	2.75			
	North	158	97.55	2.97			
	Total	530	97.50	2.83			
Weight (Kg)	South	106	73.61	22.28	0.634	0.594	NS
	East	105	69.83	22.34			
	West	161	70.88	19.78			
	North	158	71.80	20.60			
	Total	530	71.49	21.03			
Height (Cm)	South	106	168.23	7.03	0.975	0.404	NS
	East	105	167.61	6.28			
	West	161	168.76	6.52			
	North	158	168.89	6.41			
	Total	530	168.46	6.54			
BMI (kg/m <sup>2</sup> )	South	106	25.86	7.03	0.626	0.598	NS
	East	105	24.76	7.47			
	West	161	24.82	6.42			
	North	158	25.05	6.53			
	Total	530	25.08	6.79			

The *p*-value for the one-way ANOVA tests (according to school area) for age was *p*=0.929; for standard waist/age was *p*=0.978, for weight *p*=0.594, for height *p*=0.404 and for BMI *p*=0.598. Since the *p*-value > 0.05 in all cases, this means that there is no significant difference for the following parameters: age, standard waist/age, weight, height and BMI according to the school area.

Comparing the physical activity of adolescent students from the four different geographical areas were studied. As shown in tables (2, 3) walking per week ranged from 2.72 to 3.1, with a total average of

2.98±2.5 without a significant difference between students from the four different areas (F=0.89, *p*=0.446), whereas the minutes walking per time was varied significantly (F= 3.64, *p*= 0.013) according to the area, and ranged from 25.29 to 33.08, with an average of 30.94±34.81. Other parameters such as, stairs use per day, Jogging/running, minutes jogging, biking, swimming and minutes swimming were non significantly varied among students from the four geographical areas, it averaged 6.91±5.69; 2.12±2.18; 24.71±33.68; 1.08±3.56 ; 0.98±5.36 and 27.98±44.42, respectively.

**Table 2.** Time in minutes spent in different types of moderate activity. One way ANOVA tests were used to compare the mean values according to the school area (total n = 530)

	Area	N	Mean	±SD	F	P-value	Significance
Walking frequency per week	South	106	3.05	2.34	0.890	0.446	NS
	East	105	3.10	2.50			
	West	161	2.72	2.47			
	North	158	3.13	2.63			

	Total	530	2.98	2.50			
Minutes walking per time	South	106	27.97	31.10	3.640	0.013	Sig
	East	105	33.08	41.81			
	West	161	25.29	28.04			
	North	158	37.28	36.81			
	Total	530	30.94	34.64			
Swimming	South	106	0.76	1.31	1.456	0.226	NS
	East	105	1.95	11.73			
	West	161	0.71	1.36			
	North	158	0.74	1.36			
	Total	530	0.98	5.36			
Minutes Swimming	South	106	30.75	42.17	1.888	0.131	NS
	East	105	35.75	53.81			
	West	161	23.98	46.20			
	North	158	25.02	35.97			
	Total	530	27.98	44.42			
Household Activity	South	106	1.27	2.03	4.897	0.002	Sig
	East	105	1.50	1.99			
	West	161	0.82	1.54			
	North	158	0.84	1.40			
	Total	530	1.05	1.73			
Min household Activity	South	106	12.99	22.87	5.310	0.001	Sig
	East	105	17.26	27.71			
	West	161	6.53	12.16			
	North	158	13.46	26.59			
	Total	530	12.01	22.91			

In addition, household activity and min household activity recorded a significant variations ( $p=0.002$ ;  $p=0.001$ , respect.) among adolescents in

different geographical areas in KSA, the values averaged  $1.05\pm 1.73$  and  $12.01\pm 22.91$ , respectively (Table 2).

**Table 3.** Time in minutes spent in different types of vigorous activity. One way ANOVA tests were used to compare the mean values according to the school area (total n = 530)

	Area	N	Mean	±SD	F	P-value	Significance
Stairs use per day	South	106	6.92	5.86	2.009	0.112	NS
	East	105	6.69	4.47			
	West	161	6.23	4.83			
	North	158	7.76	6.92			
	Total	530	6.91	5.69			
Jogging/Running	South	106	2.04	2.03	0.516	0.672	NS
	East	105	2.05	2.19			
	West	161	2.05	2.18			
	North	158	2.30	2.28			
	Total	530	2.12	2.18			
Minutes jogging	South	106	24.41	37.96	1.620	0.184	NS
	East	105	27.10	44.85			
	West	161	20.20	23.24			
	North	158	27.91	30.68			
	Total	530	24.71	33.68			
Biking	South	106	1.02	1.78	2.444	0.063	NS
	East	105	1.88	7.31			
	West	161	0.65	1.57			
	North	158	1.03	2.05			
	Total	530	1.03	2.05			

Minutes biking	Total	530	1.08	3.65	4.227	0.006	Sig
	South	106	13.08	25.81			
	East	105	18.94	53.82			
	West	161	5.54	13.20			
	North	158	10.99	24.12			
Self-defense	Total	530	11.33	30.81	0.300	0.826	NS
	South	106	0.27	1.06			
	East	105	0.36	1.13			
	West	161	0.24	0.93			
	North	158	0.28	1.00			
Minutes self Defense	Total	530	0.28	1.02	0.072	0.975	NS
	South	106	5.42	17.21			
	East	105	5.21	21.21			
	West	161	4.96	19.85			
	North	158	5.95	19.39			
Weight Training	Total	530	5.40	19.45	0.902	0.440	NS
	South	106	1.24	1.99			
	East	105	1.46	2.35			
	West	161	1.03	1.85			
	North	158	1.25	2.20			
Min weight Training	Total	530	1.22	2.09	0.436	0.727	NS
	South	106	16.94	31.49			
	East	105	12.48	23.93			
	West	161	14.41	30.50			
	North	158	15.55	31.53			
Total	530	14.87	29.80				

Moreover, the frequency of moderate activity, min moderate activity, frequency of vigorous activity, min vigorous activity, self-defense Min self-defense, weight training and minute weight training were also recorded a non-significant differences among the four

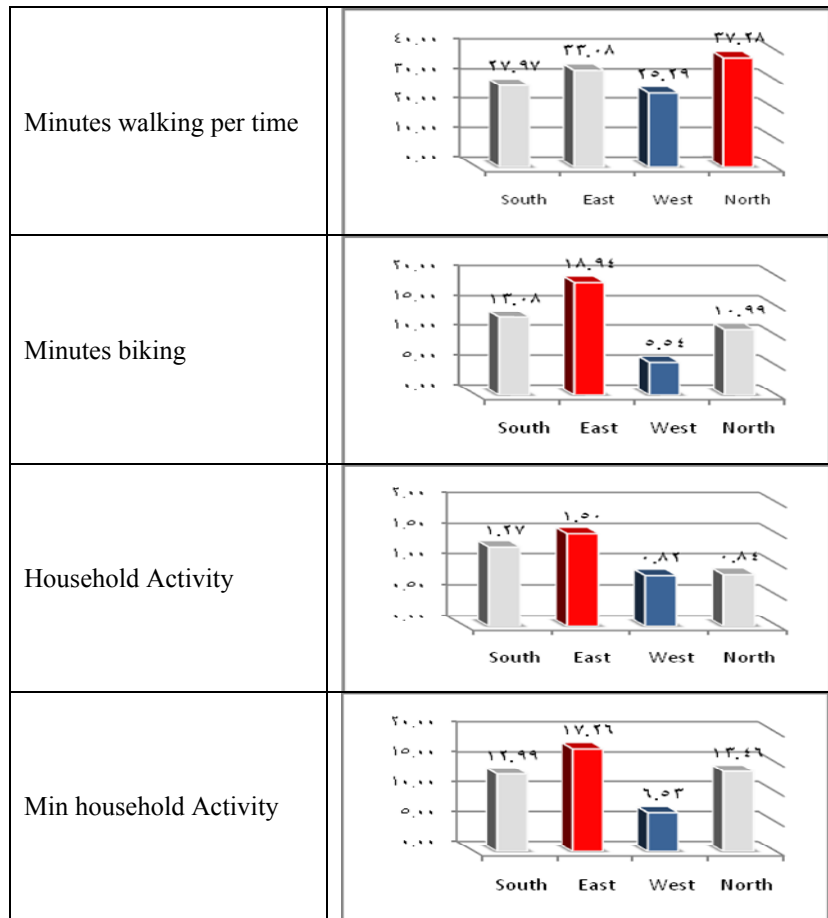
areas, it averaged,  $1.41 \pm 1.83$ ;  $25.64 \pm 32.88$ ;  $2.24 \pm 2.21$ ;  $45.90 \pm 47.40$ ;  $0.28 \pm 1.02$ ;  $5.40 \pm 19.45$ ;  $1.22 \pm 2.09$  and  $14.87 \pm 29.80$ , respectively.

**Table 4.** Time in minutes spent in different types of physical activity. One way ANOVA tests were used to compare the mean values according to the school area (total n = 530)

	Area	N	Mean	±SD	F	P-value	Significance
Moderate Activity	South	106	1.26	1.63	1.511	0.211	NS
	East	105	1.35	1.72			
	West	161	1.29	1.68			
	North	158	1.66	2.13			
	Total	530	1.41	1.83			
Min Moderate Activity	South	106	28.68	32.82	1.432	0.232	NS
	East	105	23.21	31.83			
	West	161	22.39	27.69			
	North	158	28.54	37.96			
	Total	530	25.64	32.88			
Vigorous Activity	South	106	2.29	2.03	0.504	0.680	NS
	East	105	2.43	2.47			
	West	161	2.22	2.21			
	North	158	2.09	2.17			
	Total	530	2.24	2.21			
Min Vigorous Activity	South	106	47.57	48.69	2.510	0.058	NS
	East	105	42.42	53.41			
	West	161	39.67	38.46			
	North	158	53.43	49.82			
	Total	530	45.90	47.40			



**Figure 1:** The sum of all moderate, vigorous intensity and total physical activity according to the school area.



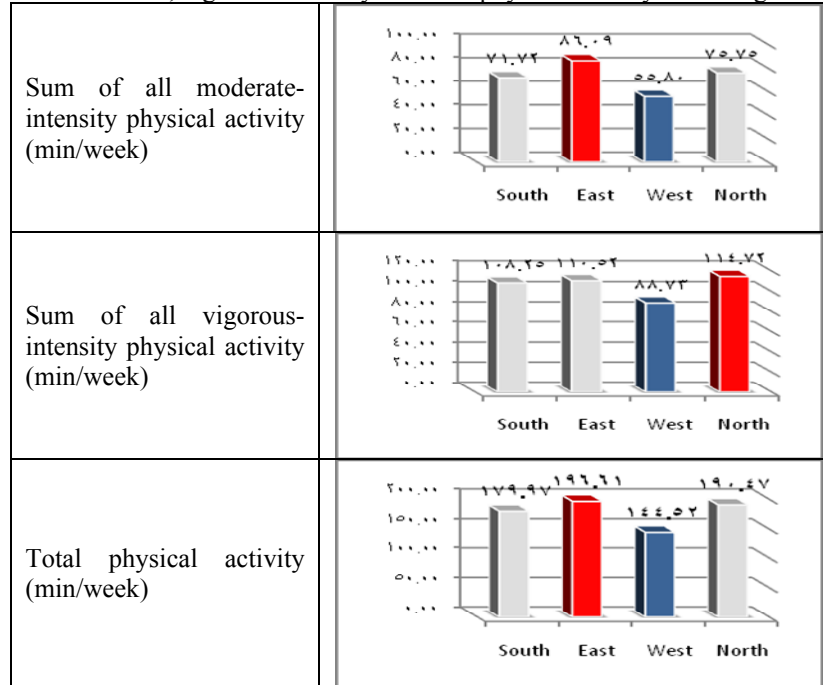
**Table 5:** The sum of all moderate, vigorous intensity and total physical activity.

	Area	N	Mean	±SD	F	P-value	Significance
Sum of all moderate-intensity physical activity (min/week)	South	106	71.72	63.75	5.013	0.002	Sig.
	East	105	86.09	70.73			
	West	161	55.80	66.32			
	North	158	75.75	62.51			
	Total	530	70.93	66.35			
Sum of all vigorous-intensity physical activity (min/week)	South	106	108.25	79.04	3.107	0.026	Sig.
	East	105	110.52	103.67			
	West	161	88.73	67.46			
	North	158	114.72	79.29			
	Total	530	104.70	81.93			
Total physical activity (min/week)	South	106	179.97	116.47	5.305	0.001	H. Sig.
	East	105	196.61	147.27			
	West	161	144.52	108.70			
	North	158	190.47	121.60			
	Total	530	175.63	123.98			

The *P*-value for the one-way ANOVA tests (according to school area) for the sum of all moderate-intensity physical activity  $p=0.002$ ; for the sum of all vigorous-intensity physical activity  $p=0.026$ , and for the total physical activity  $p=0.001$ . Since the *P*-value  $< 0.05$  in all cases, this means that

there is a significant difference for the sum of all moderate-intensity physical activity; for the sum of all vigorous-intensity physical activity, and for the total physical activity according to the school area. In all variables, the West area seem to have the lowest values, and the following figures reflect the results.

**Figure 2:** The sum of all moderate, vigorous intensity and total physical activity according to the school area.



The cluster analysis was done and yields two groups. It is noted that the first group has high mean values for each of the sum of all moderate-intensity

physical activity; the sum of all vigorous-intensity physical activity, and the total physical activity (table 6).

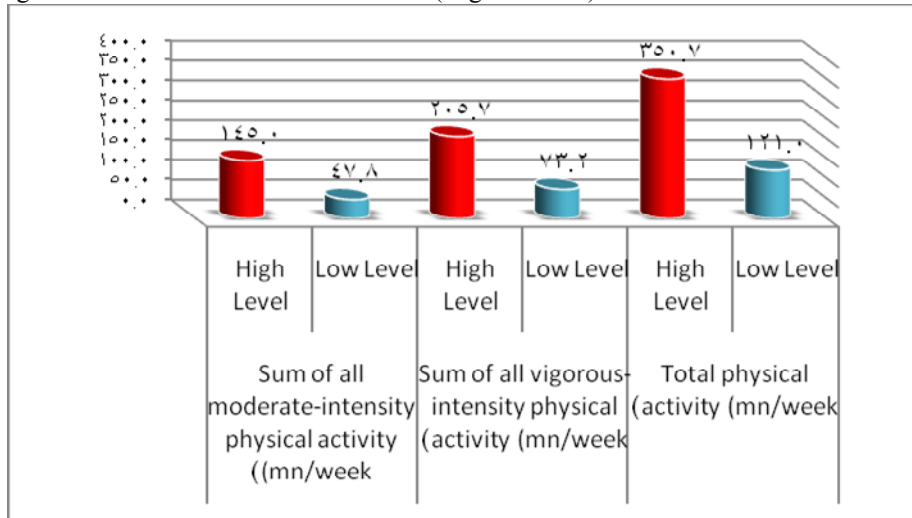
**Table 6:** The independent sample t-tests were done to test for the mean difference according to the two clusters. The results are summarized in the following table:

	Cluster Number of Case	N	Mean	SD	t	P-value	Significance
Sum of all moderate-intensity physical activity	High Level	126	145.02	82.18	18.359	0.000	H. Sig
	Low Level	404	47.82	37.85			
Sum of all vigorous-intensity physical activity	High Level	126	205.71	97.14	21.859	0.000	H. Sig
	Low Level	404	73.19	41.21			
Total physical activity	High Level	126	350.74	114.80	29.563	0.000	H. Sig
	Low Level	404	121.01	59.25			

The *P*-value for the independent sample t-tests (according to clusters) for the sum of all moderate-intensity physical activity,  $p=0.000$ ; for the sum of all vigorous-intensity physical activity,  $p=0.000$ , and for the total physical activity,  $p=0.000$ . Since the *P*-value  $< 0.001$  in all cases, this means that there is a highly significant difference for the sum of all moderate-intensity physical activity; for the sum of all

vigorous-intensity physical activity, and for the total physical activity according to the clusters and towards the high level and the following figures reflect the results.

**Figure 3:** Testing the relation between the two clusters (High and low) and the school area.



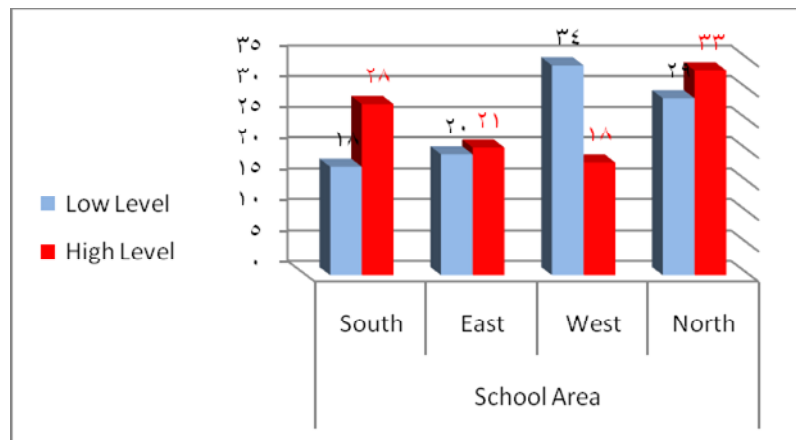
**Table 7:** Testing the relation between the two clusters (High and low) and the school area.

		Cluster Number of Cases				Total
		Low Level		High Level		
		n	%	n	%	
School Area	South	71	18	35	28	106
	East	79	20	26	21	105
	West	138	34	23	18	161
	North	116	29	42	33	158
<b>Total</b>		<b>404</b>	<b>100</b>	<b>126</b>	<b>100</b>	<b>530</b>

For testing the relation between the two clusters and the school area, the Chi-Square test was done and gives the value of  $\chi^2= 13.472$ , with  $p$ -value = 0.003 . This means that there is a significant

relationship between the two clusters and the school area, especially in the west area, where the percentage of the low level is higher than that of the high level, and the following figures reflect the results.

**Figure 4:** Testing the relation between the two clusters (High and low) and the school area.



#### 4. Discussion:

Our data of this investigation generally indicate high prevalence of physical inactivity among Saudi adolescent. This result agrees with previous studies conducted in Saudi Arabia which showed that physical inactivity is becoming more prevalent among the Saudi population (Al-Refae& Al-Hazzaa, 2001; Al-Hazzaa, 2002; Al-Hazzaa, 2004a). In addition, In Saudi Arabia, there is increasing in prevalence of overweight and obesity among children and adolescents (Abalkhail, &Shawky, 2002; Al-Hazzaa, 2007). At the same time, the proportion of inactive children and youth is high due to inability to engage most of youth in physical activity for enough duration and frequency (Al-Hazzaa, 2002; 2004a).

The decrease in the physical activity among adolescents from different areas in KSA may be attributed to changes in the life style of adolescents, which is common problem around the world. This is more so in enclosed communities specially in Arabic countries particularly Gulf region due to changes in the traffic means, increased tools of entertainments such as electronic games, videos, TV, internet etc.. All the above mentioned reasons, the adolescents spend several hours in sitting without movements which lead to obesity with different degrees and appearance of diseases not seen before in Arabic countries by this degree such as hypertension, heart diseases, diabetes mellitus among adolescents (Reilly *et al.*, 2003; Speiser *et al.*, 2005; WHO, 2010). Other investigators reported that major factors that contribute to youth inactivity in Saudi Arabia include on the use of cars rather than walking for short trip, including those to and from school (Al-Hazzaa, 2006), and the poor quality physical education programs in schools. It is well known that a comparison between physical-activity studies from different population and settings is not without reservation.

This phenomena of obesity in youth is a world health problem (Speiser *et al.*, 2005) not only during youth stage but the obesity may extend to adulthood (Guo *et al.*, 2002). Although the recommendation for physical activity in children and youth is 60 minutes of moderate to vigorous intensity per day (Strong *et al.*, 2005; Tremblay *et al.*, 2011) some health benefits can happen with 30 minutes of physical activity per day (Janssen & Leblanc, 2010).

It is believed that the high prevalence of inactivity in Saudi Arabia represents a major public health burden, as evident by the high population-attributable risk of physical inactivity compared with many industrial countries (Al-Hazzaa, 2004b). The present study reported on the prevalence of the above lifestyle factors among adolescents from different areas in the city of Jeddah. Such findings of this study

add to the existing evidence of high prevalence of physical inactivity among Saudi youth.

Findings from the European Youth Heart Study using an accelerometer for physical-activity measurements showed that the great majority of 16-year-old boys (81.9%) achieved current health enhancing physical-activity recommendations (Riddoch *et al.*, 2004). In the United States, results from the Youth Risk Behavior Surveillance indicated that only 18.4% of adolescents met these physical activity guidelines (Eston *et al.*, 2010). Furthermore, more than 52% of Greek-Cypriot children and adolescents met the physical-activity guidelines (Loucaides *et al.*, 2011). In Finland, almost half of 15-16 year old adolescents reported 60 minutes or more of total physical activity per day; however, when daily moderate- to vigorous-intensity physical activity was considered, lower proportions of the boys (23%) and girls (10%) were able to meet the recommended amount of daily physical activity (Tammelin *et al.*, 2007).

#### 5. Conclusions and Recommendations

Results from the present study confirm that low level of physical activity among Saudi adolescents, which is significantly affected by the schools areas. Programs designed to encourage increase physical activity and reduce sedentary lifestyle have been shown to improve health outcomes. A National strategy for physical activity should be developed and a preventive program should be initiated. Future researches are needed for further evaluation of other causative factors of sedentary behavior. Interventions providing knowledge, increasing consciousness of healthy benefits of physical activity and, lastly, supporting the adolescents in the adoption of healthy lifestyle.

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## AlCl<sub>3</sub>-Induced Toxicity and Oxidative Stress in Liver of Male Rats: Protection by Melatonin

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**Abstract:** Aluminum is a ubiquitous element with known toxicity for both human and experimental animals. It has been implicated in the pathogenesis of several diseases. The present study investigates the possible hepatoprotective role of melatonin in modulating the toxicity and the oxidative stress induced by chronic exposure to aluminum chloride (AlCl<sub>3</sub>) in the liver of male rats. 40 male rats were divided into four groups (10 rats each): vehicle control group treated with alcoholic saline, AlCl<sub>3</sub> group treated with 20 mg/kg of AlCl<sub>3</sub>, melatonin group treated with 5 mg/kg of melatonin, and melatonin+AlCl<sub>3</sub> group treated with the previous doses of both AlCl<sub>3</sub> and melatonin. Rats were treated orally once daily for 30 consecutive days. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bilirubin, total lipids, total cholesterol, triglycerides, glucose and total proteins were measured in the plasma to assess the liver functioning. Liver specimens were also collected for histopathological examination and also for assessment of hepatic level of malondialdehyde (MDA), reduced glutathione (GSH) in addition to the activity of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT). The results showed that the oral administration of AlCl<sub>3</sub> caused significant ( $p < 0.05$ ) increases in the plasma level of the ALT, AST, ALP, total bilirubin, total lipids, total cholesterol, triglycerides and glucose while the level of total proteins was found to be decreased. Moreover, AlCl<sub>3</sub> induced oxidative stress as indicated by a significant increase in the level of MDA with a concomitant decrease in the GSH content as well as in the activity of GPx, SOD and CAT in the liver tissue. Histological examination for liver sections revealed marked necrosis and degeneration of hepatocytes, centrilobular necrosis, congestion of the central vein, vacuolization of cytoplasm, infiltration of inflammatory cells, dilatation and congestion of the blood sinusoids. Pretreatment with melatonin in AlCl<sub>3</sub>-treated rats alleviated the previously mentioned alterations in the biochemical and oxidative stress parameters and restored their values toward the normal value of the control group. Moreover, it improved to a large extent the histological changes induced by AlCl<sub>3</sub> in such a way that more or less normal architecture of the liver was observed. Therefore, the data obtained in the present study confirmed the deleterious effects of AlCl<sub>3</sub> in the liver. Moreover, it can be concluded that these effects could be overcome or, at least, significantly minimized by the administration of melatonin.

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**Key words:** Aluminium chloride, Toxicity, Oxidative stress, Melatonin, Liver, Rat

### 1. Introduction

Aluminium (Al) is ubiquitous in the environment, it makes up close to 8% of the Earth's crust by weight. It is released to the environment either naturally through weathering and erosion processes or from various anthropogenic sources. This element has a myriad of uses in daily life throughout the world and in particular in developing countries. It is alloyed with other metals and used in many industries including ship building, electrical building and motor vehicle industries. Exposure to Al is almost inevitable, humans are frequently exposed to Al by the inhalation of ambient air and the ingestion of food and water (Soni *et al.*, 2001). Consumption of processed food and water purified using Al-containing additives is the main route for this metal to enter the human body (Newairy *et al.*, 2009). Another source of exposure is the use of Al-containing compounds such as antiperspirants, cosmetics, internal analgesics, anti-ulcerative

medications, astringents, and antacids (Zhou and Yokel, 2005). Industrial waste water and particulate matters emitted from cement-producing factories also contain high amount of Al which results in environmental pollution (Fatima *et al.*, 2001).

Aluminum has the potential to be toxic for both human and animals. It was included in the priority list of hazardous substances identified by The Agency for Toxic Substances and Disease Registry (ATSDR, 2007). It accumulates in various mammalian tissues such as the kidneys, liver, heart, brain and is related to cardiotoxicity, nephrotoxicity, neurotoxicity and hepatic dysfunctions (Reinke *et al.*, 2003). Intoxication with Al exacerbate reactive oxygen species (ROS) formation (Bondy *et al.*, 1998) and has been found to cause oxidative damage of lipids, proteins and DNA (Gonzalez *et al.*, 2007; Newairy *et al.*, 2009). Salts of Al may inhibit enzymes like acid and alkaline phosphatases, phosphodiesterase and phosphoxydase (Ochmanski

and Barabasz, 2000). The toxicological effects of Al in humans include encephalopathy (Alfrey *et al.*, 1976), bone disease (Ward *et al.*, 1978), anemia (Short *et al.*, 1980). Furthermore, Al is possibly a contributing factor in the development of Alzheimer's disease (Campbell, 2002).

Melatonin (5-methoxy-N-acetyltryptamine) is a natural compound of almost ubiquitous occurrence. Its presence has been demonstrated in all major taxa of organisms, as far as tested, including bacteria, unicellular eukaryotes, algae, fungi and invertebrate animals (Hardeland and Fuhrberg, 1996). Moreover, several studies dealt with melatonin in edible plants (Reiter and Tan, 2002). It is relatively nontoxic and highly lipophilic molecule that can easily cross cell membranes and blood-brain barrier (Hardeland, 2005). It displays a wide spectrum of metabolic and physiological effects including hypothermic (Dolberg *et al.*, 1998), analgetic, cardio- and neuroprotective effects (Lagneux *et al.*, 2000), anti-apoptotic activity (Sainz *et al.*, 1999) and antidepressive (Ergün *et al.*, 2008). Melatonin acts as a regulator for many physiological functions such as endocrine rhythm, antigonadotropic effects and stimulation of the immune system (Csernus and Mess, 2003). Melatonin is a very powerful endogenous antioxidant with a known ability to remove ROS and reactive nitrogen species (Mahieu *et al.*, 2009). Moreover, metabolites of melatonin have been found to protect tissues against oxidative damage generated by a variety of toxic agents and metabolic processes (Tan *et al.*, 2007). Melatonin upregulates several antioxidant enzymes, most frequently, glutathione peroxidase (Barlow-Walden *et al.*, 1995) and sometimes glutathione reductase (Liu and Ng, 2000). It is also essential for avoiding radical formation through downregulation of prooxidant enzymes, in particular nitric oxide synthases (Storr *et al.*, 2002).

Therefore, the present study firstly aimed to investigate the possible  $\text{AlCl}_3$ -induced changes in enzyme activities, oxidative stress biomarkers and some biochemical parameters in the plasma and liver of male rats. Since there is always need for a successful therapeutic approach that might inhibit the initiation and progression of diseases, the present study also evaluates the potential hepatoprotective effect of exogenous melatonin in ameliorating these possible alterations.

## 2. Material and Methods

### 2.1. Chemicals:

All chemicals used in the present study were of analytical grade and were purchased from Sigma-Aldrich Chemical Co. (St Louis, Mo, USA).  $\text{AlCl}_3$  was dissolved in physiological saline while melatonin was dissolved just before use in a vehicle composed

of 1% ethanol (96%) and 99% saline. All kits used in the present study were the products of Biodiagnostic Co. (Egypt), Biosystems CO. (Spain) and Randox Laboratories (United Kingdom).

### 2.2. Animals and Experimental Protocol:

Forty male albino Sprague-Dawley rats (weighing 170-190 gm) were purchased from the animal house of the High Institute of Public Health, Alexandria University, Alexandria, Egypt. Rats were housed in stainless steel cages (5 rats/cage). The animals were kept under controlled conditions of 12 h light-dark cycle, room temperature of 22-25°C, relative humidity of 40-60%. The animals were allowed free access to standard pelleted food and water. After two weeks of acclimatization to the laboratory conditions, rats were randomly divided into 4 experimental groups (10 rats in each) as follows: Group 1 (vehicle control): was administered alcoholic (1%) saline, Group 2 ( $\text{AlCl}_3$ ): given  $\text{AlCl}_3$  at a dose of 20 mg/kg bw (1/20  $\text{LD}_{50}$  of  $\text{AlCl}_3$  as reported by Krasovskii *et al.*, 1979), Group 3 (Melatonin): administered melatonin alone at a dose of 5 mg/kg bw, Group 4 (Melatonin+ $\text{AlCl}_3$ ): given the previous doses of melatonin and  $\text{AlCl}_3$  in groups 3 and 2. Doses were administered orally by gavage (since the main route of human exposure is the oral) once daily for 30 consecutive days. Melatonin was administered at 18:00, 30 minutes before the administration of  $\text{AlCl}_3$  since it is rapidly metabolized (Vakkuri *et al.*, 1985). The doses of  $\text{AlCl}_3$  and melatonin were calculated according to the animal's body weight before treatment. All rats were handled in accordance with the standard guide for the care and use of laboratory animals.

### 2.3. Blood Sampling:

At the end of the experimental duration, rats were fasted overnight, with free access to water. Under light anesthesia with diethyl ether, rats were sacrificed by cervical decapitation and the blood was collected into heparinized tubes. The collected blood was then centrifuged at 4000 rpm for 15 minutes, the obtained plasma was stored at -20°C till analysis.

### 2.4. Preparation of Liver Homogenate:

One gram of the liver tissue was washed in ice-cold isotonic saline containing 1 mM EDTA. The tissues were then homogenized separately in 8 mL of cold buffer (50 mM potassium phosphate buffer, pH 7.4, containing 1 mM EDTA) using a Potter-Elvehjem homogenizer at 4°C. The crude tissue homogenate was then centrifuged at 8,000 rpm for 15 min at 4°C and the supernatant was removed and kept at -20°C for estimation of malondialdehyde (MDA), reduced glutathione (GSH) and the activity glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT).

## 2.5. Biochemical Assays:

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined colorimetrically according to the method of Reitman and Frankel (1957). Alkaline phosphatase (ALP) was assayed according to the method described by Belfield and Goldberg (1971). Total bilirubin was assayed according to the method of Walter and Gerade (1970).

MDA, as a marker for lipid peroxidation (LPO) was measured colorimetrically in liver homogenate according to the method of Ohkawa *et al.* (1979). The non-enzymatic antioxidant GSH in liver was determined by the method of Ellman (1959).

The activity of GPx was determined as described by Paglia and Valentine (1967). According to the method of Nishikimi *et al.* (1972), the activity of SOD was assayed. The CAT activity in the liver was assayed according to the method of Aebi (1984).

Total Lipids were assayed according to the method of Zollner and Kirsch (1962). Total cholesterol was determined after enzymatic hydrolysis and oxidation according to the method of Richmond (1973). Triglycerides were measured using the method of Fossati and Principle (1982).

Glucose level in the plasma was measured according to the method of Trinder (1969). Total protein was assayed in plasma and liver homogenate according to Lowry *et al.* (1951).

## 2.6. Histological Examination:

For histological examinations, small pieces of the liver were quickly removed and fixed in 10% neutral buffered formalin solution. Following fixation,

specimens were dehydrated in graded ethanol, embedded in wax, sectioned to 5 microns thickness. The sections were stained with Haematoxylin and Eosin (Banchraft *et al.*, 1996) and examined under Olympus BX41 light microscope.

## 2.7. Statistical Analysis:

Data were analyzed using Statistical Package for Social Science (SPSS/Version 17.0) software. Significance was calculated using one-way analysis of variance (ANOVA). Values at  $p < 0.05$  were considered statistically significant.

## 3. Results

### 3.1. The Activity of Liver Function Enzymes in the Plasma

Data presented in Table 1 denoted that the administration of  $AlCl_3$  at a dose level of 20 mg/kg for 30 days induced a significant ( $p < 0.05$ ) increase in the activity of ALT, AST, ALP and the level of bilirubin as compared to the vehicle control group (by 83.1%, 80.8%, 51.3% and 144.2% respectively) indicating hepatic damage caused by  $AlCl_3$ . No change in the activity of these liver marker enzymes and in the level of total bilirubin was observed in rats administered melatonin alone as compared to the vehicle control group. Exogenous melatonin, when administered 30 minutes prior to  $AlCl_3$ , demonstrated a potent effect in protecting rats against  $AlCl_3$ -induced liver damage as evidenced by the reduction in the activity of these liver function biomarker enzymes compared to the  $AlCl_3$  group (Table 1).

**Table 1.** The activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and the level of total bilirubin in the plasma of rats treated with  $AlCl_3$  or melatonin or both.

Experimental group	Parameter			
	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (mg/dl)
Control	45.6±2.65	78.2±4.10	162.3±8.21	0.86±0.06
$AlCl_3$	83.5±5.10 <sup>a</sup>	141.4±7.64 <sup>a</sup>	245.5±10.63 <sup>a</sup>	2.10±0.10 <sup>a</sup>
Melatonin	43.0±3.85	74.5±3.89	157.6±5.82	0.80±0.04
Melatonin + $AlCl_3$	52.2±2.10 <sup>b</sup>	92.1±5.71 <sup>b</sup>	180.3±6.40 <sup>b</sup>	1.05±0.06 <sup>b</sup>

- Values are expressed as means ± SE,  $n=10$  for each experimental group.

- <sup>a</sup> Significant difference from control group ( $p < 0.05$ ). - <sup>b</sup> Significant difference from  $AlCl_3$  group ( $p < 0.05$ ).

### 3.2. Hepatic MDA Level and GSH Content

Results presented in Table 2 indicated that the level of MDA, an end product of LPO, was significantly ( $p < 0.05$ ) increased in  $AlCl_3$ -treated rats as compared to the vehicle control group. Conversely, hepatic GSH content showed significant ( $p < 0.05$ ) reduction in rats treated with  $AlCl_3$  at a dose of 20 mg/kg bw as compared to the vehicle control group. Administration of melatonin alone resulted in an insignificant increase in the level of GSH when

compared to the vehicle control group. Pretreatment with melatonin in  $AlCl_3$ -intoxicated rats restored the values of MDA and GSH toward the normal value of the control (Table 2).

### 3.3. The Activity of GPx, SOD and CAT in the Liver

The change in the activity of the enzymatic antioxidants namely GPx, SOD and CAT in liver of control and experimental animals are shown in Table 2. The oral administration of  $AlCl_3$  was associated with a significant ( $p < 0.05$ ) decrease in



the activities of these radical scavenging enzymes by 57.5%, 50.2% and 56.6% respectively compared to the vehicle control. Rats treated with melatonin alone showed an insignificant increase in the measured antioxidant enzymes compared to the vehicle control group. Administering melatonin prior to AlCl<sub>3</sub> enhanced the enzymatic antioxidative status as demonstrated by the significant ( $p < 0.05$ ) increase in the activity these enzymes in the liver in comparison with that of AlCl<sub>3</sub> group.

### 3.4. Level of Some Biochemical Parameters in the Plasma

The administration of AlCl<sub>3</sub> for 30 days was associated with some alterations in the biochemical

parameters in the plasma of rats in different experimental groups as shown in Table 3. Total lipids, total cholesterol, triglycerides and glucose showed a significant ( $p < 0.05$ ) increase (by 42.8%, 121.6%, 81.2% and 84.7% respectively) as compared to the vehicle control group. On the other hand, total proteins showed significant ( $p < 0.05$ ) decrease by 26.2% as compared to the vehicle control group. Administering melatonin alone did not induced significant changes in these biochemical parameters. The toxic effect of AlCl<sub>3</sub> was alleviated to a large extent by the pretreatment with melatonin as shown by restoring the values of these altered parameters toward the value of the control group (Table 3).

**Table 2.** Changes in the level of malondialdehyde (MDA), reduced glutathione (GSH) content, protein content and the activity of the antioxidant enzymes glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase (CAT) in liver homogenate of rats treated with AlCl<sub>3</sub> or melatonin or both.

Experimental group	Parameter					Protein (mg/g tissue)
	MDA (nmol/mg protein)	GSH (nmol/mg protein)	GPx (U/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)	
Control	0.34±0.03	47.26±2.42	74.10±3.01	68.71±3.81	58.50±3.10	156.9±4.2
AlCl <sub>3</sub>	3.11±0.24 <sup>a</sup>	18.71±1.17 <sup>a</sup>	31.45±1.84 <sup>a</sup>	34.22±2.56 <sup>a</sup>	25.40±2.85 <sup>a</sup>	113.5±3.3 <sup>a</sup>
Melatonin	0.40±0.04	51.15±3.21	79.51±4.30	74.10±3.30	63.75±3.40	160.0±6.2
Melatonin +AlCl <sub>3</sub>	0.68±0.08 <sup>b</sup>	38.32±2.83 <sup>b</sup>	63.18±3.52 <sup>b</sup>	58.81±3.92 <sup>b</sup>	47.80±3.51 <sup>b</sup>	143.7±5.0 <sup>b</sup>

- Values are expressed as means ± SE,  $n=10$  for each experimental group.

- <sup>a</sup> Significant difference from control group ( $p < 0.05$ ).

- <sup>b</sup> Significant difference from AlCl<sub>3</sub> group ( $p < 0.05$ ).

**Table 3.** Changes in the concentration of total lipids, total cholesterol, triglycerides, glucose and total proteins in the plasma of rats treated with AlCl<sub>3</sub> or melatonin or both.

Experimental group	Parameter				
	Total Lipids (mg/dl)	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)	Glucose (mg/dl)	Total Proteins (mg/dl)
Control	365.2 ± 9.4	81.32±3.11	74.6±2.60	97.43±3.83	6.91± 0.05
AlCl <sub>3</sub>	521.5±12.1 <sup>a</sup>	180.25±7.3 <sup>a</sup>	135.2±6.65 <sup>a</sup>	179.97±7.27 <sup>a</sup>	5.10±0.07 <sup>a</sup>
Melatonin	352.1±10.2	74.60±4.49	77.5±3.85	102.43±4.30	7.00±0.09
Melatonin +AlCl <sub>3</sub>	400.4±12.4 <sup>b</sup>	102.31±5.39 <sup>a</sup>	91.64±4.10 <sup>b</sup>	110.77±1.97 <sup>b</sup>	6.51±0.10 <sup>b</sup>

- Values are expressed as means ± SE,  $n=10$  for each experimental group.

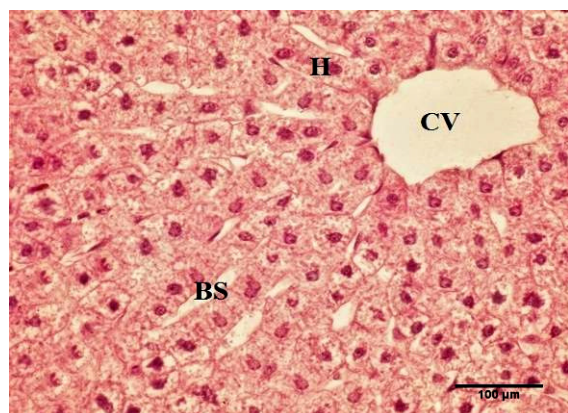
- <sup>a</sup> Significant difference from control group ( $p < 0.05$ ).

- <sup>b</sup> Significant difference from AlCl<sub>3</sub> group ( $p < 0.05$ ).

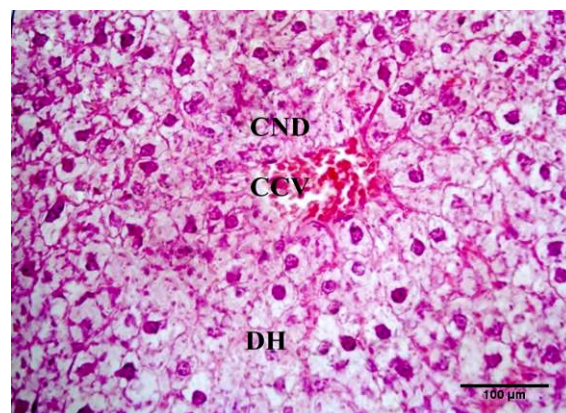
### 3.5. Histopathological examinations:

Microscopical examination of liver sections from the vehicle control group showed no evidence of histological abnormalities. The examination revealed regular hepatocytes architecture with distinct central vein, polygonal hepatocytes arranged in strands running radially from the central vein with blood sinusoids in between these hepatic strands (Figure 1). On the other hand, liver sections from rats administered AlCl<sub>3</sub> showed distorted liver architecture. Marked necrosis and degeneration of hepatocytes, centrilobular necrosis and congestion of the central vein (Figure 2), vacuolization of hepatocytes, dilatation and congestion of the blood

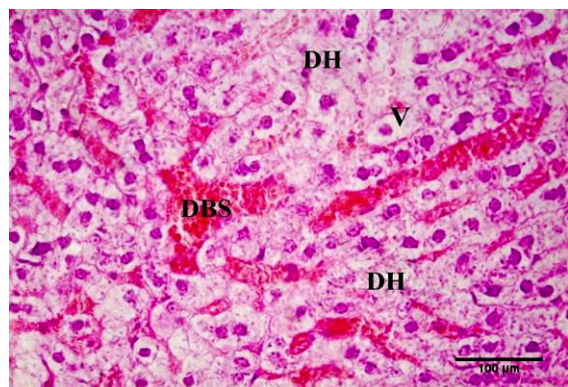
sinusoids (Figure 3) in addition to infiltration of inflammatory cells (Figure 4) were observed. No histological changes were observed in the liver of rats treated with melatonin alone indicating non-toxic effect of melatonin (Figure 5). Administration of melatonin at a dose of 5 mg/kg prior to AlCl<sub>3</sub> improved to a large extent the hepatic damage induced by AlCl<sub>3</sub> as indicated by less degeneration and necrosis of hepatocytes, minimal vacuolization of hepatocytes, disappearance of congestion in the central vein and the blood sinusoids. The examined sections revealed more or less normal architecture of the liver (Figure 6).



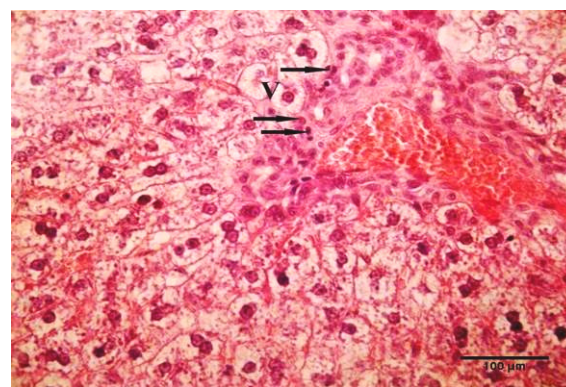
**Figure 1:** Liver section from vehicle control group showing normal histological structure: central vein (VC), hepatocyte (H), blood sinusoid (BS) (H&E x 400).



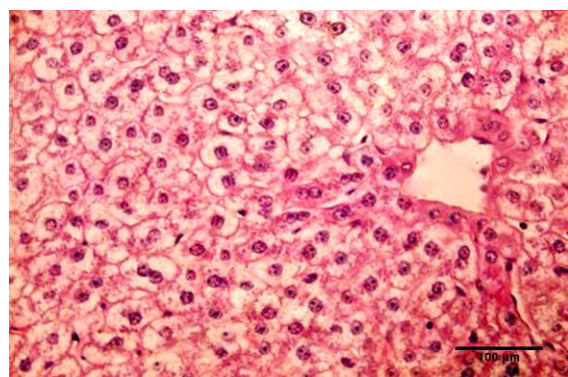
**Figure 2:** Liver section from AlCl<sub>3</sub>-treated group showing congestion of central vein (CCV), centrilobular necrosis and degeneration (CND) and degeneration of hepatocytes (DH) (H&E x 400).



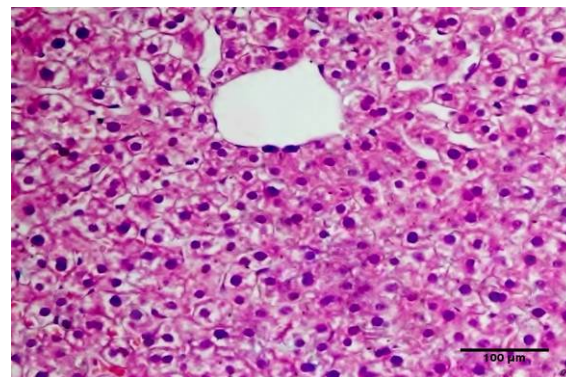
**Figure 3:** Liver section from AlCl<sub>3</sub>-treated group showing dilatation and congestion of blood sinusoids (DBS), vacuolization of cytoplasm (V) and degeneration of hepatocytes (DH) (H&E x 400).



**Figure 4:** Liver section from AlCl<sub>3</sub>-treated group showing infiltration of inflammatory cells (arrows) and vacuolization of cytoplasm (V) (H&E x 400).



**Figure 5:** Liver section from melatonin group showing nearly normal liver structure (H&E x 400).



**Figure 6:** Liver section from AlCl<sub>3</sub>+melatonin group showing less pathological changes and improved liver architecture (H&E x 400).

#### 4. Discussion

Aluminum is broadly used in daily life throughout the world. The present study investigated the potential protective effects of melatonin against

the possible toxic effects of AlCl<sub>3</sub> on the liver male rats through the evaluation of some biochemical parameters and enzymes in the plasma in addition to LPO and oxidative stress in this organ.

Exposure to high concentrations of Al can result in its accumulation in the liver and in turn to alterations in the liver function (Nikolov *et al.*, 2010). Transaminases are intracellular enzymes, released into the circulation after damage and necrosis of hepatocytes (Sallie *et al.*, 1991). The current study revealed a significant increase in the activity of ALT and AST in the plasma of  $AlCl_3$ -intoxicated rats which may be a sign of impaired liver function. The obtained data are in agreement with the earlier work of Chinoy and Memon (2001) and El-Demerdash (2004). They found that exposure to  $AlCl_3$  caused necrosis to the liver with the subsequent release of AST from the injured hepatic cells to the plasma. Plasma ALT level increases when cellular degeneration occurs which in turn indicates the existence of liver diseases. ALP is a membrane-bound enzyme related to the transport of various metabolites so it is a sensitive biomarker for liver disease (Lakshmi *et al.*, 1991). In the present investigation,  $AlCl_3$  caused a significant elevation in the activity of ALP (Table 1). This observation is in agreement with the earlier findings of Ochmanski & Barabasz (2000) and El-Demerdash (2004). Furthermore, Esmaili *et al.* (2009) reported a similar increase in the activity of ALP and they attributed it to severe damage to cell membranes or increased permeability of plasma membrane. Cell necrosis and subsequent release of membrane bound enzymes into the blood circulation following Al intoxication could also explain its elevated level (Bansal *et al.*, 2005). The impaired liver function observed in the present study is thought to be mediated by LPO which causes damage to cell membranes. LPO of cell membranes leads to loss of membrane fluidity, changes in membrane potential and an increase in membrane permeability (Nehru and Anand, 2005), all of which lead to leakage of the enzymes from the liver cells. Moreover, Al shows high affinity for phosphate groups and binds to the phospholipid head through electrostatic forces, which may induce conformational changes in the lipid bilayer of the plasma membrane (Martin, 1986). Another possible mechanism for the observed elevation in the enzymes may be liver dysfunction and disturbance in the biosynthesis of these enzymes which all are indicative of liver damage and thus impaired liver function. The observed increase in the concentration of total bilirubin in  $AlCl_3$  group may be due to the fact that Al exposure can result in its accumulation in the liver which can be toxic to the hepatic tissue at high concentrations and may lead to the increase in bilirubin level (Gonzalez *et al.*, 2007). Pretreatment with melatonin in  $AlCl_3$  intoxicated rats resulted in improvement in ALT, AST, ALP and total bilirubin indicating improved liver function and protection

against  $AlCl_3$  toxicity. The hepatoprotective effect of melatonin can be attributed to its antioxidative effect. Melatonin may hamper  $AlCl_3$ -induced LPO and in turn protects cellular membrane integrity from oxidative damage and prevents the leakage of hepatic enzymes (Carla *et al.*, 2009).

Reactive oxygen species (ROS) have been implicated in the etiology of several diseases including atherosclerosis, inflammatory conditions, neurodegenerative diseases, cancer, diabetes mellitus, renal, pulmonary, cardiac diseases and the process of aging (Young and Woodside, 2001). It has been reported that the toxic effects associated with Al are related to the generation of ROS which results in oxidative damage to cellular lipids, proteins and DNA (El-Demerdash *et al.*, 2004). LPO, as one of the main manifestations of oxidative damage, has been found to be a major contributor in the pathogenesis of many diseases and in the toxicity of many xenobiotics (Anane and Creppy, 2001). The data obtained in the present study revealed elevation in the level of hepatic MDA as a marker for LPO. Several investigations reported that Al has the ability to potentiate iron-mediated LPO (Oteiza, 1994; Ohyashiki *et al.*, 1998). Disruption in mineral balance through replacing iron ions with Al and the subsequent increase in the amount of the free iron can explain the increased LPO. The free iron ions have a strong catalytic power to generate highly reactive hydroxyl radicals from hydrogen peroxide through Fenton's reaction (Ward *et al.*, 2001). These radicals are able to initiate LPO and cellular damage.

The body has antioxidative mechanisms to stabilize oxidative molecules, control lipid oxidation and keep these radicals in balance. When free radicals are generated, the body defends itself from these radicals by endogenous antioxidants (Halliwell, 1994). However, when endogenous antioxidants become insufficient in defense against oxidants, exogenous antioxidants are needed to restore the balance. Endogenous antioxidants, either non-enzymatic (as GSH) or enzymatic (as GPx, SOD and CAT) represents the first line of defense against free radical damage and are crucial for preventing or at least slowing the incidence and progression of diseases (Jacob, 1995). The findings of the present study showed that the administration of  $AlCl_3$  at a dose of 20 mg  $AlCl_3$ /kg induced a status of oxidant/antioxidant imbalance as indicated by the increased MDA level with a concomitant depletion in GSH content and in the activity of GPx, SOD and CAT in the liver tissue. These findings are consistent with previous studies that reported Al intake to be related to alteration in the activity of tissue antioxidant enzymes and promotion of oxidative stress (Yousef, 2004; Nehru & Anand, 2005;

Newairy *et al.*, 2009).  $AlCl_3$  induced free radicals and it may inhibit the antioxidant defense system. Orihuela *et al.* (2005) reported that high doses of Al are able to induce free radicals and resulted in reduced GSH synthesis by decreasing glutathione-synthase activity. The reduction in the activity of GPx, SOD and CAT observed in the present study may be attributed to the reduced synthesis of these enzymes due to higher intracellular concentrations of Al and/or accumulation of free radicals. The inhibition of the activities of these enzymes may also be referred to the effect of Al in declining the expression of mRNA of endogenous antioxidants (Gonzalez *et al.*, 2007). The data obtained in the present study illustrated that melatonin restored the oxidant/antioxidant balance as reflected by the decrease in MDA level and the stimulation of the antioxidants GSH, GPx, SOD and CAT in the liver. Melatonin and its metabolites are powerful antioxidants that can directly detoxify free radicals species *via* electron donation (Allegra *et al.*, 2003; Reiter *et al.*, 2007). It can also defend indirectly against oxidative damage by repair of the antioxidant system through enhancing the activities of a variety of antioxidative enzymes like GPx, SOD, and less commonly CAT (Rodriguez *et al.*, 2004).

Findings of the present study showed that oral administration of  $AlCl_3$  induced a significant increase in plasma level of total lipids, total cholesterol and triglycerides (Table 3). Accumulation of  $AlCl_3$  in the liver may lead to a disturbance of lipid metabolism and in turn to the reported elevation in lipid profile. As mentioned above, administration of  $AlCl_3$  resulted in increased LPO and loss of membrane integrity which might be important determinants of altered lipid metabolism and are closely associated with hyperlipidemia and/or hypercholesterolemia in many animals and human studies (Sarin *et al.*, 1997; Yousef, 2004). Al has been found to significantly affect various membrane-bound enzymes which confirm the assumption of loss of membrane integrity (Newairy *et al.*, 2009). Pretreatment with melatonin improved the altered level of total lipids, total cholesterol and triglycerides in the plasma of rats intoxicated with  $AlCl_3$ . This anti-hyperlipidemic effect of melatonin may be primarily attributed to its antioxidant activity and the protection of cellular membrane integrity from Al-induced oxidative damage (Carla *et al.*, 2009). Another possible mechanisms for the effect of melatonin on lipid profile may be its action on the gastrointestinal tract and the inhibition of cholesterol and triglycerides uptake, the augmentation of endogenous cholesterol clearance mechanisms through increasing the activity of cholesterol-degrading enzymes and/or its effect on thyroid hormones which in turn affect lipid

metabolism (Chan and Tang, 1995; Wakatsuki *et al.*, 2001).

In view of the current data, the administration of  $AlCl_3$  at a dose of 20 mg/kg resulted in an increase in the blood glucose level which may indicate disruption in carbohydrate metabolism. The observed increase in the blood sugar level in the present study is in agreement with the results of El-Demerdash (2004), Shati and Alamri (2010). This  $AlCl_3$ -induced hyperglycemia may be attributed to enhanced breakdown of liver glycogen and its subsequent increased glucose production (Yousef, 2004 and Newairy *et al.*, 2009). Moreover, it has been proposed that oxidative stress is a major pathogenic link to both insulin resistance and the dysfunction of the pancreatic  $\beta$ -cells through the formation of amyloid proteins, which not only prevents the release of insulin into the circulation, but also destroys the insulin secreting  $\beta$ -cells (Hayden, 2002). Pretreatment with melatonin in the intoxicated rats improved the hyperglycemic effect of  $AlCl_3$ . Melatonin has been found to influence glucose homeostasis (Reis *et al.*, 1996). The effect of melatonin on glucose may be explained by modification of insulin secretion and/or change in cell sensitivity to insulin. Melatonin acts on pancreatic islets and induces regeneration and/or proliferation of  $\beta$ -cells in pancreas which leads to a decrement in blood glucose in diabetic rats (Kanter *et al.*, 2006).

The data of the present work showed that Al intoxication caused a significant decrease in the plasma protein content. This result concord with the previous results of Chinoy and Memon (2001) and Newairy *et al.* (2009). The observed decrease in protein content might be attributed to higher intracellular concentration of Al in the liver which could result in reduced protein synthesis as well as reduced enzymes of protein synthesis (Tripathi *et al.*, 2009). Free radicals may also be implicated in the observed decline in protein content since exposure to the free radicals leads to protein fragmentation, protein peroxides generation, enzymatic oxidation and degradation of proteins (Albendea *et al.*, 2007). Treatment with melatonin before  $AlCl_3$  significantly ameliorated the decline in the plasma protein content probably by scavenging the free radicals and improving the antioxidative status and in turn the process of protein synthesis.

## 5. Conclusion

In conclusion, the present study indicated that oral administration of  $AlCl_3$  at a dose of 20 mg/kg daily for 30 days caused hepatic dysfunction, increase in lipid peroxidation and decline in the activity of antioxidant enzymes in the liver. It also

induced histological changes in the liver which all are attributed to free radicals production and oxidative stress. Administration of melatonin at a dose of 5 mg/kg 30 minute prior to AlCl<sub>3</sub> intoxication minimized its harmful effects and protected the liver against its toxicity which may be due to the role of melatonin as an antioxidant. Therefore, supplementation with melatonin may be useful as a hepatoprotective therapy in cases of intoxication with aluminium.

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## The Impact of Tax Ratio on Environmental performance in Iran; with Emphasis on Sustainable Economic Development

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**Abstract:** The aim of this paper is considering the impact of tax ratio on environmental performance in Iran at 1960-2009 periods. For do it, I have used regression analysis. Result of cointegration test indicates that there is a long run relationship between environmental performance and tax ratio. Estimation results indicate that tax rate has a negative effect on CO<sub>2</sub> emission, so increasing in tax rate increases environmental performance. Energy consumption and economic growth have a positive effect on pollution. So these variables have a negative effect on environmental performance. So, taxation policies could improve environmental performance in Iran.

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**Keywords:** Tax Ratio, Environmental performance, Sustainable Development, Iran

### 1. Introduction

Tax on individuals and firms is based on the quantity of incomes from economic activities in Iran. Tax is taken by Iran Tax Affairs Organization based on rights of Iran. Many factors effect on taxation as situation, firm, quality of services and so on. Taxation in Iran generates particular unease among foreign firms because they appear to be arbitrarily enforced – tax bills are initially based on 'assumed earnings' calculated by the Finance and Economy Ministry according to the size of the company and the sector in which it operates. Factors such as the quality and location of a company's offices are also widely believed to have an impact on tax assessment. The Environmental Performance Index (EPI) is a method of quantifying and numerically benchmarking the environmental performance of a country's policies. This index was developed from the Pilot Environmental Performance Index, first published in 2002, and designed to supplement the environmental targets set forth in the U.N. Millennium Development Goals.

Pearce (1991), Repetto et al. (1992), Nordhaus (1993) or Grubb (1993) among others argue that it is possible to improve tax efficiency by means of an ETR, while others, as Bovenberg and Mooij (1994) argue that this is not possible, in general, because environmental taxes are likely to increase, rather than reduce, previous distortions. Parry (1995) points out the relevance of choosing a partial equilibrium or a general equilibrium approach to answer this question. Partial equilibrium models do not take into account the interactions between environmental taxes and previous distortions, and these effects tend to cause the double dividend to hold in partial equilibrium

models but not in general equilibrium models. This is because the environmental tax eventually falls on labor income, so that labor taxes and emission taxes distort the labor market in a similar way. However labor taxes are more efficient from the levying point of view because environmental taxes also distort the relative prices between polluting and non-polluting goods, which erodes the tax base. So, from a non-environmental point of view, emission taxes are likely to cause a larger excess of burden.

Notwithstanding, the economic literature also describes some mechanisms that may cause a strong double dividend, or an employment double dividend to happen in a general equilibrium framework. An ETR could facilitate wage moderation and the reduction of labor market distortions in a situation in which imperfect competition has led to excessively high wages (Brunello, 1996; Carraro et al., 1996). Bovenberg (1994) and Carraro and Soubeyran (1996) show that, if the initial tax system is suboptimal from a non-environmental point of view, an ETR can simultaneously reduce pollution and unemployment. We can conclude that opportunities to get a double dividend typically arise when there exist some market failures or some imperfections in the tax system (see also Bovenberg and Goulder, 2002). For a survey on ETR and the double dividend, see Mooij (1999) or Goulder (1995). Given the difficulties to obtain clear-cut theoretical conclusions, it makes sense to perform an empirical analysis to test the economic effects of a specific reform in a selected country or region, by means of a suitable applied model. A number of authors, like Bovenberg and Goulder (1996), Bye (2000), Dessus and Bussolo (1998), Wender (2001), Xie and Saltzman (2000) or Yang (2001), have used



Computable General Equilibrium (CGE henceforth) models to assess the economic effects of an ETR. These models perform a disaggregate representation of all the activity sectors and the equilibrium of all markets, according to basic microeconomic principles. In Spain, Gomez-Plana et al. (2003), Labandeira et al. (2003) and Manresa and Sancho (2005) use CGE models to simulate the effect of environmental tax reforms nationwide. The aim of this paper is considering the impact of tax ratio on environmental performance in Iran. For do it, I have used regression analysis. This paper is organized by four sections. The next section is devoted to research method. In section 3, empirical results are reported. Final section is devoted to conclusion.

## 2. Material and Methods

I have used the following model for considering the impact of tax rates on environmental performance:

$$CO_2\text{Emmission}_t = \alpha + \beta t_t + \delta X_t + \varepsilon_t \quad (1)$$

$CO_2\text{Emmission}_t$  is index of environmental performance,  $t_t$  is tax ratio or the share of tax incomes in GDP,  $X_t$  is vector of control variables as population, GDP growth and energy consumption (oil consumption per capita).  $\varepsilon_t$  is error term.

Data is collected by World Development Indicator (WDI) of 2011.

### 1.2. Unit Root Tests

If an OLS regression is estimated with non-stationary data and residuals, then the regression is spurious. To overcome this problem the data has to be tested for a unit roots (i.e. whether it is stationary). If both sets of data are I (1) (non-stationary), then if the regression produces an I(0) error term, the equation is said to be counteracted. The most basic non-stationary time series is the random walk, the Dickey-Fuller test essentially involves testing for the presence of a random walk.

$$y_t = y_{t-1} + u_t \quad (2)$$

Although this has a constant mean, the variance is non-constant and so the series is non-stationary. If a constant is added, it is termed a random walk with drift. To produce a stationary time series, the random walk needs to be first-differenced:

$$\Delta y_t = u_t \quad (3)$$

### 2.2. Augmented Dickey-Fuller (ADF) Test

The Dickey-Fuller test is used to determine if a variable is stationary. To overcome the problem of autocorrelation in the basic DF test, the test can be augmented by adding various lagged dependent variables. This would produce the following test:

$$\Delta y_t = (\rho - 1)y_{t-1} + \alpha_i \sum_{i=1}^m \Delta y_{t-i} + u_t \quad (4)$$

The correct value for  $m$  (number of lags) can be determined by reference to a commonly produced information criteria such as the Akaike criteria or Schwarz-Bayesian criteria. The aim being to maximize the amount of information. As with the DF test, the ADF test can also include a drift (constant) and time trend.

Common criticisms of these tests include sensitivity to the way the test is conducted (size of test), such that the wrong version of the ADF test is used. The power of the test may depend on:

-The span of the data, rather than the sample size. (This is particularly important for financial data)

-If  $\rho$  is almost equal to 1, but not exactly, the test may give the wrong result.

-These tests assume a single unit root I (1), but there may be more than one presents I (2).

-If the time series contains a structural break, the test may produce the wrong result.

### 3.2. Engle-Granger test for Cointegration

To test for cointegration between two or more non-stationary time series, it simply requires running an OLS regression, saving the residuals and then running the ADF test on the residual to determine if it is stationary. The time series are said to be cointegrated if the residual is itself stationary. In effect the non-stationary I(1) series have cancelled each other out to produce a stationary I(0) residual.

$$y_t = \beta_0 + \beta_1 x_t + u_t \quad (5)$$

Where  $y$  and  $x$  are non-stationary series. To determine if they are cointegrated, a secondary regression is estimated:

$$\Delta u_t = -0.56u_{t-1} \quad (6)$$

(0.10)

This produces a t-statistic of  $-5.60$ . If the critical value for this model is  $-2.95$  (for example), we would reject the null hypothesis of non-stationary time series and conclude the error term was stationary and the two variables are cointegrated.

### 4.2. The Granger Representation Theorem

According to this theorem, if two variables  $y$  and  $x$  are cointegrated, then the relationship between the two can be expressed as an error correction model

(ECM), in which the error term from the OLS regression, lagged once, acts as the error correction term. In this case the cointegration provides evidence of a long-run relationship between the variables, whilst the ECM provides evidence of the short-run relationship. A basic error correction model would appear as follows:

$$\Delta y_t = \chi_0 + \chi_1 \Delta x_t - \tau(u_{t-1}) + \varepsilon_t \quad (7)$$

Where  $\tau$  is the error correction term coefficient, which theory suggests should be negative and whose value measures the speed of adjustment back to equilibrium following an exogenous shock. The error correction term  $u_{t-1}$ , which can be written as:  $(y_{t-1} - x_{t-1})$  is the residual from the cointegrating relationship in (4).

**5.2. Johansen’s Procedure**

Intuitively, the Johansen test is a multivariate version of the univariate DF test. Consider a *reduced form* VAR of order  $p$ :

$$y_t = A_1 y_{t-1} + \dots + A_p y_{t-p} + Bx_t + u_t \quad (5A)$$

Where  $y_t$  is a  $k$ -vector of  $I(1)$  variables,  $x_t$  is a  $n$ -vector of deterministic trends, and  $u_t$  is a vector of shocks. We can rewrite this VAR as:

$$\Delta y_t = \Pi y_{t-1} + \sum_{i=1}^{p-1} \Gamma_i \Delta y_{t-i} + Bx_t + u_t \quad (6A)$$

Where  $\Pi = \sum_{i=1}^p A_i - 1, \Gamma_i = -\sum_{j=i+1}^p A_j$

The error correction model (ECM), due to Engel and Granger (1987). The  $\Pi$  matrix represents the adjustment to disequilibrium following an exogenous shock. If  $\Pi$  has reduced rank  $r < k$  where  $r$  and  $k$  denote the rank of  $\Pi$  and the number of variables constituting the long-run relationship, respectively, then there exist two  $k \times r$  matrices  $\alpha$  and  $\beta$ , each with rank  $r$ , such that  $\Pi = \alpha\beta'$  and  $\beta'y_t$  is stationary.  $r$  is called the *cointegration rank* and each column of  $\beta$  is a cointegrating vector (representing a long-run relationship). The elements of the  $\alpha$  matrix represent the *adjustment* or *loading* coefficients, and indicate the speeds of adjustment of the endogenous variables in response to disequilibrating shocks, while the elements of the  $\Gamma$  matrices capture the short-run dynamic adjustments. Johansen’s method estimates the  $\Pi$  matrix from an unrestricted VAR and tests whether we can reject the restrictions implied by the reduced rank of  $\Pi$ . This procedure relies on

relationships between the rank of a matrix and its characteristic roots (or eigenvalues). The rank of  $\Pi$  equals the number of its characteristic roots that differ from zero, which in turn corresponds to the number of cointegrating vectors. The asymptotic distribution of the Likelihood Ratio (Trace) test statistic for cointegration does not have the usual  $\chi^2$  distribution and depends on the assumptions made regarding the deterministic trends.

**3. Empirical Results**

In this section, I tested unit root test for the variables. Co2 is  $I(1)$ , Tax ratio is  $I(1)$ , GDP growth is  $I(0)$ , Oil Consumption is  $I(1)$  and population is  $I(2)$ .

**Table1. Unit Root Tests**

Null Hypothesis: D(CO2) has a unit root  
 Exogenous: Constant  
 Lag Length: 0 (Automatic based on SIC, MAXLAG=9)

	t-Statistic	Prob.*
Augmented Dickey-Fuller test statistic	-7.047771	0.0000
Test critical values:		
	1% level	-3.581152
	5% level	-2.926622
	10% level	-2.601424

\*MacKinnon (1996) one-sided p-values.

Null Hypothesis: D(T) has a unit root  
 Exogenous: Constant  
 Lag Length: 9 (Automatic based on SIC, MAXLAG=9)

	t-Statistic	Prob.*
Augmented Dickey-Fuller test statistic	-2.963534	0.0513
Test critical values:		
	1% level	-3.699871
	5% level	-2.976263
	10% level	-2.627420

\*MacKinnon (1996) one-sided p-values.

Null Hypothesis: GROWT has a unit root  
 Exogenous: Constant  
 Lag Length: 0 (Automatic based on SIC, MAXLAG=9)

	t-Statistic	Prob.*
Augmented Dickey-Fuller test statistic	-3.587297	0.0101
Test critical values:		
	1% level	-3.592462
	5% level	-2.931404

10% level	-2.603944
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\*MacKinnon (1996) one-sided p-values.

Null Hypothesis: D(OIL) has a unit root

Exogenous: Constant

Lag Length: 0 (Automatic based on SIC, MAXLAG=9)

	t-Statistic	Prob.*
Augmented Dickey-Fuller test statistic	-5.708419	0.0000
Test critical values:		
1% level	-3.639407	
5% level	-2.951125	
10% level	-2.614300	

\*MacKinnon (1996) one-sided p-values.

Null Hypothesis: D(POP,2) has a unit root

Exogenous: Constant

Lag Length: 0 (Automatic based on SIC, MAXLAG=10)

	t-Statistic	Prob.*
Augmented Dickey-Fuller test statistic	-3.426162	0.0149
Test critical values:		
1% level	-3.577723	
5% level	-2.925169	
10% level	-2.600658	

\*MacKinnon (1996) one-sided p-values.

**Table 2. Cointegration Test**

Sample (adjusted): 1975 2007

Included observations: 33 after adjustments

Trend assumption: Linear deterministic trend

Series: CO2 T GROWT OIL POP

Lags interval (in first differences): 1 to 1

Unrestricted Cointegration Rank Test (Trace)

Hypothesized No. of CE(s)	Eigenvalue	Trace Statistic	0.05 Critical Value	Prob.**
None *	0.585326	78.58336	69.81889	0.0085
At most 1 *	0.488817	49.53471	47.85613	0.0345
At most 2	0.409181	27.39080	29.79707	0.0924
At most 3	0.210651	10.02469	15.49471	0.2788
At most 4	0.065021	2.218632	3.841466	0.1364

Trace test indicates 2 cointegrating eqn(s) at the 0.05 level

\* denotes rejection of the hypothesis at the 0.05 level

\*\*MacKinnon-Haug-Michelis (1999) p-values

Table 2 indicates cointegration test for the model. Result indicates that there is a long run relationship between environmental performance and tax ratio.

**Table 3. Estimation Results**

Method: Least Squares

Date: 11/22/11 Time: 09:27

Sample (adjusted): 1973 2007

Included observations: 35 after adjustments

White Heteroskedasticity-Consistent Standard Errors & Covariance

Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	4.141045	0.642668	6.443521	0.0000
T	-3.102498	2.147938	2.375534	0.0141
OIL	0.007869	0.003696	2.128912	0.0416
GROWT	0.028545	0.013807	2.067450	0.0474
POP	-2.48E-08	2.01E-08	-1.233085	0.2271
R-squared	0.789508	Mean dependent var	4.528254	
Adjusted R-squared	0.761443	S.D. dependent var	1.093238	
S.E. of regression	0.533963	Akaike info criterion	1.714583	
Sum squared resid	8.553494	Schwarz criterion	1.936776	
Log likelihood	-25.00521	Hannan-Quinn criter.	1.791284	
F-statistic	28.13083	Durbin-Watson stat	0.473486	
Prob(F-statistic)	0.000000			

Table 3 indicates estimation results. Results indicate tax rate decrease on CO2 emission, so increasing in tax rate increases environmental performance and Sustainable Development. Energy consumption and economic growth have a positive effect on pollution. So these variables have a negative effect on environmental performance. R-squared is 79%. This means that the model has a suitable goodness of fit.

**4. Discussions**

Tax bills are initially based on 'assumed earnings' calculated by Iran Tax Affairs Organization according to the size of the company and the sector in which it operates. Factors such as the quality and location of a company's offices are also widely believed to have an impact on tax assessment. The Environmental Performance Index (EPI) is a method of quantifying and numerically benchmarking the environmental performance of a country's policies. This index was developed from the Pilot Environmental Performance Index, first published in 2002, and designed to supplement the environmental targets set forth in the U.N. Millennium Development Goals.

The aim of this paper is considering the impact of tax rates on environmental performance in Iran. For do it, I have used regression analysis. I tested unit root test for the variables. Co2 is I(1), Tax ratio is

I(1), GDP growth is I(0), Oil Consumption is I(1) and population is I(2). Result of cointegration test indicates that there is a long run relationship between environmental performance and tax ratio. Estimation results indicate that tax rate decrease CO<sub>2</sub> emission, so increasing in tax ratio increases environmental performance and suitable development. Energy consumption and economic growth have a positive effect on pollution. So these variables have a negative effect on environmental performance. So, taxation policies could improve environmental performance in Iran and this is an important on suitable development in Iran.

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**Iranian 1000-year-old short story**

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**Abstract:** Generally it is said that short story in Iran has begun by Yeki bud, yeki nabud (Once upon a time), penned by Jamalzadeh. Although this opinion seems to be true from the contemporary scholars' point of view, by studying the original story of Afshin and Bodelf in Tarikh-E Beyhaghi it is understood that Beyhaghi has selected the framework of telling stories in stories and has narrated the story in a way that owns all the features and elements of contemporary stories. This paper aims to display the artistic talent and writing capability of Beyhaghi in "Afshin and Bodelf" whose theme is "whilte lie." This story which has a realistic framework was accomplished by Beyhaghi and mediated by "Ismael Ben Shahab" and "Ahmed Ben Abi David" (Hero narrator). The overall story is moving deductively. In this drama-like story, there are two kinds of internal and external descriptions. One of the most important features of Afshin and Bodelf is its dramatic form. In the end, dear readers will find out that writing story in Iran is beyond imitation western contemporary stories and is as old as a millennium.

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**Keywords:** short story, episode, theme, plot, angle of view, dramatic, hero, Ahmed, Afshin, Moetasim, scene, description

**1. Introduction**

Tarikh-E Beyhaghi is a unique historical work of Persian literature which is very important in many aspects, but it has not been comprehensively studied. The commitment of Abolfazl Dabir to true research and his detailed and inductive approach have made his work as a literary-historical masterpiece. We think that Tarikh-E Beyhaghi (*Truths and Lies* 1999) is like a novel in which different stories have been beautifully narrated and stories have been expressed within stories and have been displayed like attractive episodes one after the other. One of these episodes is the short story "Afshin and Bodelf." It should be noted that the equivalent term for "short story" in ancient literature is the term "anecdote" (Hekayat) which appears as an episode in the context of a long story and sometimes it is independent." (FAZILAT MAHMOOD, NAROOEI SEDIGHEH. 2012). Edgar Allan Poe who is one of the greatest short stories writers in the world literature and as important as Anton Chekhov, Ernest Hemingway, William A. Henry, Guy de Maupassant and etc., As the father of short story, has counted the following features for this kind of literary genre which he himself called prose tale:

- should be abridged enough to be read in half-to-two hours
- only talks about one issue
- have the same effect on the readers
- without additional terms that have no role in the story's impression or plan

- be perfect and comprehensive from the reader's point of view (Love, quoted from several sources)

Therefore, in this article it is attempted to study one of the factual stories of Tarikh-E Beyhaghi with regard to the features of modern short stories and thus to argue the existence of short stories in Iran in 1000 years ago.

**1.1. Text:**

Tarikh-E Beyhaghi covers certain historical events in detail in a narrative style and the author has applied narrative tool that is dialogue and theoretical and dramatic description. Beyhaghi was a realist historian and a political analyst who used explicit characterization (description by the narrator or one of the characters) and implicit characterization (description by action and behavior in fiction and displaying the habits and states) and also other elements of the story to express an allegorical or real story. Therefore, his work's narrative framework includes several structures or in other words it is a frame story which is a combination of core and long narratives and also short stories and episodes. In fact, these episodes, like the fact that speech brings speech, have been used like an example to analyze or explain a point or to convince the readers and to express historical events and their accuracy and to promote the overall action of a long story. The best example of this kind is the real story of Bubekr Hasiri and his son, and the story of Afshin and Bodelf is mentioned as an evidence to the saying "speech

brings speech,” so that at the end of Hasiri tale it is said: “... I have read a tale about the Caliph Moetasim which is similar to what I have narrated, but a bit more appalling, and I think it is necessary to be mentioned because the special book of history becomes more attractive with such events and the readers would enjoy it, since speech brings speech.” (Truths and Lies 1999). Unlike novels, in short stories it is not possible to describe characters entirely because of time and space limitations. Therefore there are one or at most two main characters in short stories. In short story “Afshin and Bodelf” there are three dynamic characters (Karami Mohammad Hossein 2011).

With regard to related explanations in this part, talent and writing capability of Beyhaghi in describing some elements of episode-like story “Afshin and Bodelf” will be displayed.

### 2.1. Theme:

The main theme of this story is “white lie” which prevents the murder of an innocent person; as mentioned before, this short narration approves of the main story that is the tale of “Abu Bakr Hasiri” and escape of his son and himself from the wrath of minister Ahmed Hassan Meimandi with mediation of “Bunasr Meshkan” chief of Epistle and deals with great leaders and government officials. (Truths and Lies 1999). Beginning of the story has a realistic setting. The main motive of the story is a telepathic feeling of anxiety or fear between two characters who are Moetasim, the round character and Ahmed Ebn ABi David, the protagonist. Although the main character of the story is described at the beginning of the story by Beyhaghi in a one-line statement which includes three sentences, the character of Ahmed, Moetasim and Afshin who are involved the most in the story, is processed inductively through their action and behavior; Antagonist with the help of Moetasim) achieves the ultimate goal (rescuing the innocent person from the hand of Afshin and his soldiers) after handling and fighting opposite forces. Then the story comes to an end with a natural falling and the reader feels relieved at the peak of anxiety. The most dynamic character, who tries to achieve the ultimate goal, is Ahmed Ebn Abi David. To clarify this issue, it’s better to summarize the story first:

Antagonist, a guy named Ahmed Ebn Abi Davis, vizier of Moetasim, Abbasid Caliph has insomnia a night; as he tries he cant go to sleep and he severely feels upset and nostalgic. He calls his servant and orders him to saddle his horse and the servant makes him aware of his inappropriate order; finally after going to the bathroom and washing his face and hands, he rides a donkey which was saddled and goes to the Caliph’s court. Amir who is intelligent asks

him, “Abi Abdellah why are you late? I’ve been waiting for you a long time (Truths and Lies 1999). After investigation, it becomes clear that the Caliph, has handed Abodelf (Arab commander) over to his Iranian commander, Afshin. The reason is Afshin’s demand from Caliph to take an old revenge and the caliph agrees in order to keep his promise related to defeating “Babak Khoramdin” by Afshin. Vizier makes the Caliph aware of the risks of this command. Caliph who finds he can’t do anything to change the situation resorts to his vizier and asks him to take measures to prevent Bodelf’s murdering. He goes to Afshin’s house frantically to beg him not to kill an innocent person. He can’t convince him to stop in spite of all begging, requests, contempt, promises and advice, kissing hands and bowing and planning to kiss his feet. Finally, he prevents Afshin from killing Bodelf by telling a white lie so that, if he kills innocent Bodelf, the Caliph would kill him and then he returns to the Caliph’s court. Then, Afshin goes to the court angrily to know about the reason and the truthfulness of the command quoted on behalf of Moetasim. Caliph, who is confused, approves of his vizier’s lying inevitably (although he was satisfied with the result from the bottom of his heart) and Afshin returns empty-handed. “Afshin left while his demand failed and his limbs dead.” And when Afshin returned, Moetasim asked: “Oh! Abi Abdellah why did you prefer to give an untrue message?” Ahmed Abi David says: “Oh Amir Al-Moemenin (Chief of the righteous), I don’t like to kill a Muslim and I will be rewarded and God will pardon me for telling a lie.” And refers to some verses from Quran and the prophet (peace be upon him) to approve of his own action. Moetasim laughs and says “What you did is quite right (Truths and Lies 1999). This story revolves around themes such as inequality in Arab and Ajam thoughts at the time of this historical event, unjustly bleeding, shaking ideas of Abbasid Caliphs (in the history) and more important, white lie which is a reminder of popular Persian proverb, “white lie is better than seditious truth.”

### 3.1. Point of view:

The story that happened in the past and ended is narrated by Beyhaghi, with two mediators “Ismael Ebn-E Shahab” and “Ahmed Ben Abi David,” a hero. This form of narration is called first person narration, since “in this form, the main character of the story tells his own story himself using “I,” which is not necessarily the author.” That is in this story; Ahmed Ebn-E Abi David who is the main character of the story narrates the story himself as: “One night I Woke up at Moetasim court. As I tried I couldn’t sleep and I felt upset and nostalgic. I called a servant ...” But the point is that Beyhaghi

narrates this story from the language of this hero, where he says at the beginning of the story: "I heard from Ahmed Ben Abi David" and since it is about history he has documented it as: "Ismael Shahab says I heard from Ahmed Ben Abi David \_ and this Ahmed was a man who was more magnificent than the viziers with the judge that he had ..." (Truths and Lies 1999). Therefore, the narrator is first person and at the same time the success of this hero, is the end of the story. The language of the story creates such a feeling that engages the mind very attractively. Physical movements of the narrator (Ahmed Ben Abi David) against the negative force of the story which represents the uncontrollable violence and ambition of antagonist (Afshin), narrator's modest supplication and his extraordinary request, protests and wonderful changes of the heroes' tone of voice keeps the text impenetrable to the end and engages the mind of the audience in this challenge.

In plot of the story, the arrangement of related situations near each other and the concentration of the reader on them is significant, because the lead to the formation of a whole in his mind to be united and form a single action. The first question which arises in early scenes is that "What has happened?" It is the concern of the hero narrator and then passes to another character of the story (Caliph), who is himself one of the causes of crisis and incidents in the story. Of course this crisis-making character becomes aware of his mistake and helps the protagonist to succeed. Since the matter is revealed (handing Bodelf over to Afshin to take a revenge) another important argument is raised which is asking about the "fate" of an important character of this story, Abodelf. This event and frequent actions of the narrator which is together with excitement, fear, and anxiety make the story very dynamic.

In this story, minor characters such as servant, Salam, the narrator accompanying forces, guards of Afshin's court, executioner, Moetasim's doorkeeper, etc. are not directing the audience's attention towards the most important aspects of major characters, each of them belong to their own status which, like the status of three main characters (Narrator, Afshin, and Abodelf), draw our attention to some consecutive questions. The overall movement of the story is from the part to the whole (inductive approach). Heroes are introduced and described mainly through their direct actions, except for some cases in which they are introduced by some people like the Caliph (introducing Afshin and Bodelf in second episode). This dramatic description which is idiomatically called implicit personification by the novelists is one of the strengths of the story.

Using short sentences in the text, especially from the beginning, creates a stressful atmosphere in

this story which is kept to the end of the story which is a natural falling, and the narrator's questions have made this atmosphere more exciting and stronger.

"style of this narrative story, is more similar to one kind of drama in which scenario is set by a speaker or writer based on a pre-determined adventure, and the term drama in Beyhaghi means such kind of narrative story." (Junejo Imran N. et al. 2008). In this drama-like short story, there are two kinds of description:

A. External description of characters and events B. Internal description of the heroes. Narrator-hero narrates most crucial parts of the story with monologue or struggling with himself or his own ideas or mind. The story lacks any introduction or background context and the main cause of the event – Afshin's malice towards Bodelf – is narrated by one of the main characters of the story (Caliph) who is himself a crisis-making character. The reason is that Beyhaghi didn't mean to write just a story, but he planned to extract heart of the matter from outdated tales. What sometimes makes the story similar to a drama is using a special language for the narrator (narrative) and the heroes (dialogue) and setting the scenes and displaying those behaviors in succession. One of the features of Afshin and Bodelf is its dramatic image ; because numerous scenes of this story created by Beyhaghi are ready to be converted to an attractive drama or short movie ; on the whole, there are 14 consecutive single scenes in this drama-like story including:

1. The scene of Ahmed Ben Abi David's discomfort after midnight and his dialogue with a servant named Salam
2. The continuance of his discomfort, his taking a bath and his order to the servants to saddle the horse
3. Going to the Caliph's court with a saddled donkey
4. Attending the court and asking for help from the doorkeeper and their dialogues
5. Ahmed's visiting and talking with Moetasim and planning to rescue Bodelf
6. Riding a horse from the Caliph's court to Afshin's court, asking for permission to enter and facing his doorkeepers
7. Visiting Afshin (the main part of the text) and wonderful dialogue and begging and request and changing the tone of voice from modesty to commanding
8. A short dialogue with Bodelf about his health and his companions' witness
9. Confused and hasty return to Moetasim's court, visiting the Caliph and expressing the events
10. Afshin's entering the court and arguing with Caliph and pretesting to his verdict and then returning

11. Caliph's calling on giving untrue message and Ahmed's response
12. Doorkeeper's going to Afshin's court to return abodelf
13. Returning home with Ahmed while keeping a distance
14. Bodelf's arriving at Ahmed's house and expressing his gratitude to Ahmed (end of the story)

Applying sentences which are suitable in different situations is very significant in this story; Narrator's discomfort and the main first person is expressed from the beginning of the story with very short affirmative sentences and the narrator's internal monologues. "One night at Moetasim era I woke up after midnight. As I tried, I couldn't go to sleep and I felt sad and nostalgic and I didn't understand the reason. I told myself what has happened. I called a servant who was always very close to me. His name is Salam, I ordered him to saddle the horse." (Truths and Lies 1999). Beyhaghi was able to narrate the slow passing of time with few narrative sentences very easily, while he narrates all the time that is needed for Vizier Ahmed (Protagonist) to reach to Caliph's court in detail using very short statements. Mentioning all events in detail in this setting in a short time and its description and explanation help this principle. Displaying these slow stressful moments is the pre-requirement for creating such an atmosphere and the author's favorite sense. Moreover, the scene is described objectively and "displaying" the "objective" and explicit action of the hero is another artistic advantage of this section. He said: "Oh! My Lord, it is midnight and tomorrow is not your turn, since the Caliph has said that you have to do certain task and you won't get any load and if another person is intended, it's not time to sit, I kept quiet and knew that he was right, but I felt discomfort and I was inspired that something had happened, I got up and called the servants loudly to light the candle, the I went to the bathroom and took a bath, I was worried to be on time, I put on clothes, and rode the donkey which was saddled and really I didn't know where I was going..." (Beyhaghi, pp.161,162). Detailed description and explanation of all the hero's actions, while raising expectations, contribute to introducing him and on the other hand express his modes. An important point here is the frequency of the verbs. These verbs, apparently do not answer the question which is raised in the mind of the hero and consequently in the mind of the reader so that they increase his stress; and this is the first writing art of Beyhaghi in this story. The story gradually moves faster since the time Ahmed goes to the Caliph's court. And since their visit it gets faster which is kept until the end of the story and even increases while

Ahmed is visiting Afshin. The talent of the author in this regard is that he has rarely used the conjunctive "and" so that the rhythm of the sentences is kept fast; "He said: welcome, it's open, get in. In I got, I saw Moetasim, hard thinking and doing nothing. I greeted him and he replied. He asked me, "Oh I Aba Abdillah, why are you late? I've been waiting for you a long time ... He said: "Don't you know what has happened?" "No," I said. He said: "We all belong to Him and we surely return to him. Sit down to hear." (Truths and Lies 1999).

In this scene the author expresses the occurred events through the language of one of the characters, Caliph, with a flash back and introduces to main characters, Afshin and Bodelf. Another important point in this story is using short sentences with reference verbs which have reverse effect so that it indicates a long duration of time with anxiety and stress specially when the effort to rescue the innocent accused is useless so that his status does not change at all as if nothing has been done. In this regard, the author describes the behavior of main character, Ahmed, in detail through his internal monologues, and narrates the scene of visiting Ahmed with Afshin so long by using the conjunctive "and" that it seems it lasted much longer time. Only through this way of narration the author can illustrate the real character of Afshin in a miserable scene and emphasize it and can keep it in the mind of the reader. "As Afshin saw me he got angry and his face got yellow and red from wrath and the arteries rose up from his neck. I waited patiently and read a Hadith to amuse him, so that he shouldn't tell the executioner to behead. Of course he didn't look at me, I stood up, I started to speak another way and began to admire Ajam ( Persians) and preferred Persians to Arab. For the sake of Bodelf to rescue him. And I heard nothing. I said: Oh Amir, I have come here to ask you to forgive him to me like a lord. He said angrily: I won't pardon him at all since grat Amir has handed him over to me. I kissed his shoulder again. It wasn't useful, I kissed his hand again he saw I was going to kneel down and kiss ..." (Truths and Lies 1999).

The plan or the arrangement of the events in the story is called plot which shows how and why the events happen in the story. On the other words, plot arranges and combines the events in the story so that it seems quite logical to the reader.... In fact, plot is the way of narrating events with emphasize on cause and effect which shows in every literary work the cause of every event and also the consequences of every event." Therefore the fourteen events are a plot to describe the events; and through direct dialogue between the characters the plot has developed. Regardless of internal monologue of the narrator, these dialogues begin by a character and are



interrupted by another one. This issue has made the atmosphere of the story very similar to drama: "As Afshin heard this matter, he was shocked and shaking and his limbs were dead and he said: Is this message really from our lord? I said: Yes, Have you ever heard that I'd ignore his commands? I called on my relatives to come. Thirty forty men appeared from different classes and ages. I told them: You are witness that I give the message of Amir Moetasim to this Amir Abolhassan Afshin.... Then I said: Oh, Ghasim! He said: yes? I said: Are you all right? He said: yes. I said: Are you Injured? He said: No." (Truths and Lies 1999). Mentioning the adverbs of manner in the dialogues of the characters such as "softly," "modestly," "Kindly," "hardly" helps their mental performance.

#### 4.1. Internal monologues:

Of the hero narrator have been mentioned sever times, which is another important point that offers necessary information about the scenes and characters and their narrative description; it also slows down the movement of the story. (). In the following example, the narrator's belief to Ajam (Persian) is expressed in his own self: "I preferred Ajam (Persian) to Arab although I knew it was a great sin just for the sake of Bodelf in order not to be murdered." (Truths and Lies 1999). Also the prose and diction and the style of these dialogues are remarkable which are appropriate to the heroes' era (their narrators). "Since the aim of dramatic speech is to say to be performed and as the nature of this kind of speech is the language of individuals who speak themselves, therefore it has a feature which is applied in daily life." (Dovsen, p.43). Therefore, the components of these dialogues are like the ordinary speech of the author's era, used by aristocrats. Another point which is related to drama techniques and this story is benefiting it is "suspense" which is created by raising a question in every scene and finding an answer to it in the following scene. (Dawson,S. W.1970). Therefor all scenes of this story have "rising" and "falling." This approach is more similar to a controllable internal voice (Corrigan Timothy. 2011) which is repeated like a motive and increases the emotional sense of the story:

I was thinking that I shouldn't arrive late so that they would bring Bodelf and kill him and everything would have gone (Narrator's internal question). Some questions raised in the mind of the reader with some stress which in fact attracts him to read the rest of the story are as the followings:

The forest herbs, which play important role for rural communities for example, the livestock totally dependent on them for fodder and as traditional medicines, have been hardly studied from

diversity standpoint (Singh and Singh 1987). Quantitative information on the forest floor species of the Central Himalaya region is generally lacking except for studies done by Rawat and Singh (1989), and Singh and Singh (1992). Interestingly, most of the recent major field experiments addressed questions relating to species diversity which has been carried out in grasslands. But forest herbs of the Himalayan region remain poorly studied. In the present study we investigate herb species richness (spermatophyte) in terms of taxonomical diversity and species composition in relation to oak and pine forests in Central Himalayan forests.

- Would the ideas of vizier Ahmed useful after consulting with the Caliph?
- What would be the Caliph's reaction to the lie of vizier Ahmed?
- What would be the Caliph's reaction to Afshin's protest?
- What would be the Caliph's response?
- What factor would inhibit Afshin from killing Bodelf?
- How would vizier Ahmed justify his lie?
- What would finally happen to Bodelf?

Another point is the questions which are raised in every scene of the story which suspends the reader and attracts him to continue reading the story to the end which is more due to the narrator's tone of voice and is called "dramatic irony." (Dawson,S. W.1970) The story of Afshin and Bodelf has the following features of short story apart from its comprehensive book of Tarikh-E Beyhaghi:

1. It has one main character. (Ahmed Ben Abi David)
2. This character is displayed in one main action.
3. It is a short story that according to Allen Poe can be read in one session (including 7 pages).
4. The falling of the story has the most prominent role (rescuing an innocent person from death).

If we compare all these distinctive features of this story, we conclude that: Afshin and Bodelf "is a short story in which the author illustrates a main character in an event through an organized and brief plot and this work on the whole induces a sinfle effect." (John Green. 1989). Since Beyhaghi has written a history not a story, the mentioned factors are not originated from his dreams but are due to the procedures that he has applied to make the story of history more impressive. He has taken advantage of making up and trimming speech which is Beyhaghi's exclusive art and distinguishes him from his era historians and even contemporary historians. Now with regard to these points and with reference to Allen Poe's point of view about short story, this section of Beyhaghi's book can be classified as a

short story and he can be called as one of the first authors of short story of Persian language.

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## Multi-grade classes teaching methods

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**Abstract:** This paper aims to deal with the definition of multi-grade classes, its history in Iran and the world, learning objectives of these classes, the causes of using these classes in Iran, teaching style in these classes considering the presence of special students, problems, limitations, and disadvantages of this type of classes, the need for this type of classes regarding the demographic situation, roles and responsibilities of teachers in these classes, and the goals of these classes. This study tries to slightly help the teachers to manage multi-grade classes better and resolve part of education system in relation to these classes. We hope that this small effort will be accepted by God, education system, dear and hardworking teachers of educations system and multi-grade classes.

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**Keywords:** Multi-grade classes; teaching methods; learning objectives

### 1. Introduction

Every country and nation tries to educate their children by their own education system, because it is believed, experience and knowledge have proven it, and that no nation can move ahead of its culture. Hence, in every country including Iran, there are regions in which the number of students in each grade is very small and occasionally there are only one or two students in each grade. According to the importance of this issue, education systems has established multi-grade classes in these region in order to educate the children and follow the educational justice(Wesmood, P (1997). The need for multi-grade classes is an inevitable reality in education system of our country. Environmental adverse conditions, shortage of students, manpower shortage, insufficient educational space, voluntary or forced migration, various financial and economic problems, distant villages, and lack of sufficient resources are factors that restrict the possibility to establish educational units with single-grade classes (Collingwood, I. (1992). Hence, familiarity of officials with multi-grade classes, the course of their development, principles of learning in these classes, and professional features and limitations of multi-grade classes is essential. On the other hand, expansion of multi-grade classes from elementary schools to guidance schools requires more extensive research in order to identify and assess the problems of the existing problems of teaching in these classes. Providing more appropriate teaching methods, identifying and resolving the educational problems of teachers and holding on-the-job training courses, and comparative-analytical study of multi-grade classes in various countries around the world can be also effective in this regard.

### 1.1. Definition of multi-grade classes:

A class which is attended by students of two or more grades, taught by one teacher, and held in one classroom is called a multi-grade class and multi-grade education is the opportunities in which students varying in age and ability all are trained in one class. Organizationally, multi-grade education can be done in many forms, from a set of common educational grades under the supervision of one teacher to educational units that are essentially devoid of any grading. Therefore, multi-grade schools are the opposite of traditional schools in which the students of each grade are educated in a separate classroom and with a special teacher. One of the requirements of success in multi-grade education is a set of teaching techniques and skills that make it possible to teachers to effectively teach the students varying in age and educational abilities (McEwan, P.J. (1998).

### 1.2. The history of multi-grade classes:

#### A) In Iran:

With a little research in the history of education it can be found that multi-grade classes are as old as the onset of collective education. Before qualitative and quantitative changes in education and the development of new methods (classical and planned education in a modern form), education system was an education tailored to the current needs and values at any time and influenced and overwhelmed by governments, regimes, societies, sects, and even families. Isa Sedigh, in his book named "History of Iran's Culture", points out that the history of multi-grade education date back to the years 250 to 260 (According to Solar Hijri calendar). He mentions schools as one of the educational institutions in the past and writes, "School was an institution at which Quran reading and reading and

writing were taught. Schools were held in mosques, teacher's home, shops, and so on. Individual education was used in schools. The teacher first taught the older student and then asked them to teach smaller students what they had learned (WGTP (2005). After the development of modern education system, schools were considered informal and their number decreased with the establishment of a number of elementary schools until 1976 that there was no record of schools in official statistics.

#### **B) In the world:**

Prior to the Industrial Revolution and urbanization, multi-grade education was the most widespread form of education in North America. Oversight and assistance system, in which older and more advanced students helped other students and monitored their progress, are and old tradition. Multi-grade education is still of great importance in many parts of North America and Europe, especially in villages and towns around big cities. Multi-grade education provides valuable services in counties and villages of Scotland, Canada, France, the U.S., and Scandinavian countries (Little, A. (1995)). There are more than 420000, 20000, and 1540 multi-grade single-room schools in China, Indonesia, and Malaysia. 8% of schools in Philippines and 37% of elementary schools in India have only one or two teachers. In 22% of schools in Mexico, students of 6 grades are taught in a single classroom. Multi-grade education is very common in the Pacific island countries, in which small groups isolated from other people live. The number of such schools (Multi-grade classes) is increasing in most European countries (Murdoch, D., & Schiller, J. (200).

A new survey in the Netherlands shows that 29% of all elementary grades have students varying in age. Another study in the North West of England also indicates that there is a kind of grouping of various ages in 66% of studied schools (Mohr, N., Dichter, A. (2001).

#### **1.3. Objectives of multi-grade classes:**

- 1- Providing a complete education with increasing the accessibility to it in rural and sparsely populated areas
- 2- Continuity of educational services in small towns that are faced with shortage of students
- 3- Efficient use of limited resources
- 4- Improving the quality and effectiveness of education

#### **1.4. Benefits of multi-grade classes:**

- 1- Multi-grade education is an efficient and useful method to provide basic education in sparsely populated areas
- 2- This method has a high efficiency in applying limited educational resources such as trained teachers, educational space, etc.

3- Surveys and evidence indicate that this method gets students improved at math, foreign language, and science.

4- Girl students have more access to educational places and facilities in this method.

5- In this type of classes, students are taught how to learn and how to teach.

6- Teacher and student can develop a constructive and dynamic relationship and students can even help their teacher in evaluating and applying appropriate educational strategies.

7- Multi-grade education gives the opportunity to student varying in age to interact with each other (Nielsen HD.et.al (1993).

#### **1.5. The causes of using multi-grade classes in Iran:**

- 1- Adverse environmental conditions
- 2- Distant villages and specific geographical factors
- 3- Shortage of must-be-educated students in some villages and sparsely populated towns
- 4- Shortage of educational space
- 5- Shortage of manpower
- 6- Voluntary or forced migration of villagers to big cities

Hence, in order to cover all must-be-educated children and continue their education, establishment of education units with multi-grade classes, particularly in rural and sparsely populated regions is unavoidable.

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Hence, in order to cover all must-be-educated children and continue their education, establishment of education units with multi-grade classes, particularly in rural and sparsely populated regions is unavoidable.

B) Teaching a single course to all grades in a session one after another: In this practice, all grades are taught a single course in a session one after another. For example, the teacher teaches to the students of grade 1 and assigns their tasks and then start teaching math to the students of grade 2 and so it continues.

Combination of axial and collective methods: This combination of the two previous methods.

It is noteworthy to say that all three methods flexible enough.

#### **1.7. Problems, limitations, and disadvantages of multi-grade classes:**

1- The intellectual and material attention and investment required to train teachers for these classes have not been spent and always the weakest and most inexperienced teachers have been appointed to these classes.

2- Non-specialist persons allow themselves to be involved in planning for these classes.

3- Some special problems of these classes are as follows:

A) On top of these problems is the lack of time, as there are 123 hours-courses in a 5-grade class that all of them should be covered in this short time.

B) Low experience and knowledge of teachers in this school on details.

C) In appropriate age composition of students (varying from 6 to 15 years old), while all are present in one class and are encouraged or punished in one way.

D) Family involvements of students

E) The presence of boy and girl students in one class

F) Lack of educational space and classrooms which is followed by some problems such as impossibility to divide the grades for teamwork independent teaching of teachers, impossibility to observe health issues, limitations in use of educational tools and edutainment.

G) Lack of a rich environment and lack of coordination between school and family education.

H) Outspoken critics of teachers due to poor living and educational conditions

I) Lack of basic educational equipment

Some of the limitations and peripheral problems of rural schools are as follows:

A) Some teachers don't live in the village they are teaching it and have to commute and sometimes are inevitably absent.

B) Closure on Thursdays

C) Heavy responsibilities of teachers in multi-grade classes

D) Disciplinary matters of learners in terms of emotional deficiencies, malnutrition, poor health, and other living conditions

E) Seasonal migration of some families to other regions

F) Dropout for reasons like migration, monetary factors, etc.

G) Lack of supervision and cooperation of families in educational issues of their children

H) Teachers' dissatisfaction because of tough conditions

I) Non-compliance with the programs and class discipline

J) Educational weakness and backwardness due to the problem in previous years

K) Lack of information of teachers on how manages and teaches in these classes.

### **1.8. A framework for curriculum reform:**

1- Determining the curriculum

2- Reviewing the curriculum

3- Analyzing the minimum learning potentials

4- Identifying the relevant and shared skills and also direct and indirect teaching skills

5- Adapting the curriculum with local conditions

6- Providing appropriate educational tools and edutainment

7- Testing and implementing the adapted curriculum

### **1.9. Roles and responsibilities of teachers in multi-grade education and their general traits:**

Teachers of multi-grade classes should be experienced and specialist and show a high level of some traits such as sacrifice, forgiveness, compassion and kindness, patience, diligence, competency, commitment, mastery of local language, having advanced skills in communication, efficient and effective management in the classroom, enthusiasm to work, and great perseverance.

### **1.10. The necessity of multi-grade classes:**

Due to increased number of multi-grade classes and covering a significant number of elementary learners in such classes, continuous change and displacement of teachers, inability of parents to participate and cooperate in education, lack of time for teachers to plan, new approach of primary school textbooks and range of activities and also in order to cooperate with teachers in the process of teaching-learning, improve the education, prevent academic failure in multi-grade classes, increase the rate of progress, and strengthen the incentive of teachers to teach in these classes, the use of educational and edutainment software and CDs seems to be necessary. It is vital to provide the substrate for deepening in learning and academic promotion of students in rural and underserved regions through promoting and developing the culture of using computers and educational CDs and also dedicating to extracurricular activities.

### **1.11. The objectives of multi-grade classes:**

1- Efforts to establish equal educational opportunities, especially in rural schools

2- The use of computers as an adjunct teacher

3- Optimal use of time in teaching the learners and saving the time of such classes

4- Enrichment of educational programs and activities and deepening the learning process

5- Accelerating the teachers and students access to large volumes of scientific and educational data, information, and materials in a limited time

6- Improving the education in multi-grade classes and preventing the academic failure

7- Making the educational environment attractive and providing educational facilities and tools commensurate with the progress of science and

knowledge in underserved and sparsely populated areas.

8- Increasing the knowledge retention of learners

#### **1.12. Way of holding, reasons and necessities:**

A multi-grade class, based on the number of grades and students, can be held in different ways. For example, a couple more than one blackboards can be used in classrooms so that students can focus better or student can be place in form of a "U". Placement and sorting of student depend on teaching style and teacher's enthusiasm to use different teaching methods and the following 2 reasons can be mentioned for its necessity:

1- Students reach cognitive and social development in these classes and also these classes reduce the anti-social behaviors of students.

2- Administratively, as the number of students in these grades is less, it is economically more affordable for the government.

Running multi-grade classes is more sensitive and difficult than single-grade classes, because teachers should deal with students varying in age and try to make the class fun for everyone. Teachers and students need to have a friendly relationship with each other and the teacher should believe in abilities of students and emphasize on their positive points and avoid punishment and other things such as befooling the students or threatening them by the leverage of mark. In order to teach different grades in a classroom, one of the grades should be considered as the center of teaching and assign tasks for other grades.

#### **1.13. Disadvantages of multi-grade classes:**

- 1- Shortage of time
- 2- Distraction of students
- 3- Students varying in age
- 4- Mismatch between textbooks and learning abilities of students in these classes
- 5- Shortage of trained and qualified teachers to manage such classes
- 6- Neglecting multi-grade education by teacher training centers and lack of training for teachers to deal with the problem of such classes

Along with all these disadvantages, the following benefits can be cited for multi-grade classes:

- 1- Cognitive, social, and emotional growth of students
- 2- Providing the substrate for learning some courses like math
- 3- Common teaching of some concepts
- 4- Reviewing the material for higher-grade students and providing the substrate fore lower-grade students to learn.

#### **1.14. Your general comments about class management and interaction with students in multi-grade classes:**

Class management may differ by different methods of teaching. Students are graded and their tasks are assigned. In some cases, the teacher should use individual teaching and testing. When the teacher assigns the tasks, student interacts with each other two by two and also with the teacher. For example, there is an interaction between the teacher and students when the teacher is asking them questions.

#### **1.15. The arrangement of multi-grade classes:**

Depending to the interests and tastes of teacher, teaching style, learning abilities of students, and other conditions, the arrangement of these classes may vary. However, the following two forms are more common. U-shaped method: This form helps the teacher to have a complete domination on classroom, especially the blind spots, so that all can easily have a eye contact with each other and with the teacher. In addition, the teacher can help the student who have auditory problems and get them involved in the process of teaching-learning by sitting them in the front rows and closer to the blackboard.

With paying a little attention to above-mentioned order, it can be found that this arrangement also has many advantages over conventional arrangements and is known as "triangular arrangement", provided that the number of rows is not much. If the number of learners is high, these rows are shortly repeated one after another and are pulled towards the back of classrooms (Nielsen, Dean. (1995).

## **2. Discussions**

It can be concluded from the material discussed in this paper that given the circumstances in some regions like underserved areas, the use of multi-grade classes seems to be necessary. However, these classes have many problems, limitations, shortcomings, weaknesses, strengths, and benefits that the officials of education system should try to minimize weaknesses, limitations, and problems and reinforce the strengths.

Here are some of proposed solutions:

- 1- The need to strengthen teacher training centers and including a course titled "Multi-grade classes" in the curriculum of teachers.
- 2- The use of assistant and semi-professional teachers, from local community if possible, to help teachers.
- 3- The use of peer teachers. This means that student who has higher abilities can help the teacher to teaching other students.
- 4- The use of parents who can participate more effectively in the process of teaching. This can

develop a close relationship between community and school.

5- Providing special facilities and accommodations for these teachers and increasing their salary.

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## Top management of innovative competitiveness in industries

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**Abstract:** Competitiveness is a key criterion for evaluation of success degree of countries, industries, and firms in political, economic, and commercial fields of competition. With rapid advances and changes in the world, innovative competitiveness has gained a special importance to speed up the development of the economy of industries. One of the consequences of globalization is the emergence of cross-regional economies which have increased their gross domestic production (GDP) compared with other countries and caused changes in production techniques in industries in order to produce new and modern products. Hence, some authors believe that regional changes and political and economic relations of countries with each other can speed up and develop the innovative competitiveness. Porter's Diamond model, conceptual frameworks, and the review of industries of developing countries were used in this study to precisely identify the components of competitiveness.

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**Keywords:** Competitiveness, industry, Porter's Diamond model

### 1. Introduction

The challenge caused by global competition has increased the pressure on organizations to improve their skills, capabilities, and the quality of their services and products. Principles of total quality management (TQM) have been widely accepted throughout the world as a tool to improve organizational performance and the nature of organizations and deal with particular challenges of markets (Tan, P.K.L. 1997). Asian financial crisis which started in the middle of 1997 with Thailand's financial-cash crisis triggered a chain that affected almost all Asian countries. This crisis and conflicting changes that are raised have had several points and hints for improvement of economic and developmental situation in organizations and industries of Asian countries and caused extensive changes in the structure of these organizations. These changes have significantly affected the movement of total quality management. Principles of total quality management have been widely accepted throughout the world as a tool to improve organizational performance and the nature of organizations and deal with particular challenges of markets.

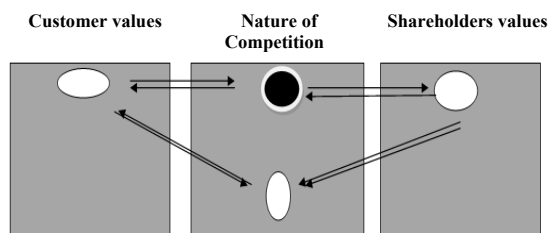
Pattern of competitiveness through considering the enablers creates the foundations of improvement in different dimensions of organization and establishes a logical consistency in each of them by a systematic approach. On the other hand, it creates a synergy in organizational enablers by integrating the complex of enablers, resulting in improved capability of employees, increased quality, and access to new markets. Consequently, profitability of organization can be guaranteed by ruling out the competitive intelligence and driving strategies on organization.

### 1.1. Competitiveness:

Competitiveness has been defined as ability and desire to enter the competition. Economically speaking, Michael Porter defines it as efficiency and how an organization or nation uses its own human resources, capital, and natural resources (Porter, M. (1998a)). In a small scale, competitiveness is referred to a value that a product creates for the customer compared with a competing product which depends on two factors including the amount of utilities met from ownership or possession of a product for the customer and cost of ownership or possession of a product for the customer. Distinction in each of these two factors makes the organization competitive. If an organization sets its strategy based on offering more special and superior utilities than competitor to the customer, it has chosen the strategy of distinction and if an organization follows the strategy of offering non-competitive prices, it has chosen the strategy of cost leadership. Strategy of distinction improves the competitiveness using more utilities, while cost leadership strategy makes the organization competitive compared with competitor by reducing inutilities (costs) (like Chinese clothing manufacturers). Each of these strategies will make the organization competitive in a competitive market. In addition to proper response to competitive market of product and service, a successful organization should be accountable to capital market. Costs of these strategies for producer and operating profit of company for the shareholders must be acceptable for this purpose. Competitiveness has been defined as ability and desire to enter the competition. Economically speaking, Michael Porter defines it as efficiency and how an organization or nation uses its



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### 1.2. The concept of competitiveness:

Views of various experts and scholars show that there is no uniform definition and interpretation of competitiveness. Generally, competitiveness can be defined as competencies and capabilities that a business, industry, region, and country possesses and can maintain them to produce a high return rate in manufacture parameters in the international competition and keep their human resources in a relatively high status. In other words, competitiveness is the ability to increase market share, profitability, increased the value added, and remain in a fair international market for a long period (Castellacci Fulvio. 2008). Competitiveness is a combination of assets and processes. Assets are whether blessings like natural resources or created by human like infrastructures. Processes convert the

assets to economic benefits derived from product sales to customers and thereby create competitiveness. Competitiveness can be viewed from another perspective which is the sources of competitiveness. Competitiveness sources can be divided into three categories including technology, organization, and human resources. Competitive advantage obtained from human resources is more durable and sustainable than other competitive advantages and more time is required to allow competitors to imitate such competitive advantages.

### 1.3. Porter's Diamond model:

Michael Porter is a Harvard Business School professor and one of the most active researchers in the field of competitiveness studies. He wrote the book "Competitive Strategy" in 1980 and the book "Competitive Advantage" in 1985. Additionally, he published another book named "Competitive Advantage of Nations" in 1990, in which Diamond model is introduced. A variety of theories and models have been proposed to explain and interpret the competitiveness, Porter's Diamond model has a special importance and position among them. According to this model, four main parameters including internal factors, domestic demand conditions, relevant and supporting industries, and strategy, structure, and competition have a direct impact on competitiveness of countries and their industries. In addition, government and unforeseen events indirectly affect the competitiveness. Although this model has been the basis of many studies around the world and attracted the attention of many researchers, it has totally some weaknesses. Lack of emphasis on international issues and global markets and neglecting the displacement of production factors by multinational corporations and foreign direct investment are some these weaknesses. Additionally, government's role in underdeveloped countries is beyond an indirect effect. Hence, these points should be taken into account in assessment of competitiveness based on Porter's Diamond model.

### 1.4. The role of technology in competitiveness:

Competitiveness is a process through which any institution tries to excel others. In fact, it can be considered as an attempt by a firm, industry or country to catch up another firm, industry or country in the competition. At the international level, countries due to lack of financial, technical, and specialized resources should compete with each other in order to achieve wealth and benefit their society with prosperity. Therefore, acquisition of competitive capabilities has become one of the major challenges of countries in today's world at the international level. Various factors are prerequisite for the enjoyment of competitive power at the national and international level. These factors include standard of living, trade,

productivity, and investment. The combination of these factors determines the competitiveness of a country at the international level. Technology can play a key role in each of these factors. Necessary infrastructures for investment can be provided with the help of technology. Also, technology increases production efficiency and thereby the productivity. The following example can clarify the role of technology.

#### **1.5. Japan Wall (made of thinking and creativity):**

Geoffrey C. Lloyd, (1996), talked about the construction of Japan Wall in his memories of visiting a Japanese factory producing car clutch in 1987. Unlike the Chinese wall which is based on tyranny, this wall is built of the glorious thoughts of factory's staff. "When I was viewing visiting the many wonders in production hall of this factory, I noticed one of the walls of this hall with a length of about 50 meters and a height of 8 meters covered by papers full of notes. Curiosity forced me to ask a question about it. In response, I found that these papers were the recommendations of employees for converting the production system into CAD/CAM (Computer-Aided Design/ Computer-Aided Manufacturing) and each employee had proposed 11 written recommendations on average. Since the management believed that the success of this project owed for the proposals of employees at all levels and also in order to encourage and promote participatory management system and recommendations system, the largest longitudinal wall of this hall was adorned with the thoughts of employees. In fact, many researchers introduce the recommendations system as the main system and tool for continuous improvement and the main core of all superior systems of quality and productivity based on such observations. Brady, J.E. & Allen, T.T. (2006)) state "Probably before the Six Sigma project, you should consider a recommendation system program and run it." According to Miller, it is the pure creation of thinking in a business that makes all committed to daily improvement. Recommendations system is one of the tools of pure production which is misunderstood and misused. Luis Arroyo, the director of advanced production of Sensormatic Company and the winner of Shingo Prize that the company he is working there has recently saved 1.7 million dollars through the recommendations of 1200 employees, believes that recommendations system is a critical and important component in helping us to implement the pure production. This system makes it easier for us to do basic tasks. Currently, 91.7 of registered enterprises in Tokyo Stock Exchange are implementing the recommendations system. According to Administrative Law America, all state institutions have a specific fund and administration to

implement the recommendations system and the best offer or receives a plaque of appreciation and awards from the President of the US every year (Hahn, G.et.al. (1999).

#### **1.6. Value index:**

Competitiveness management is a conceptual model which establishes the balance between benefits and costs of supply and demand. Whenever this relation reaches the optimal point in practice, long-term success in business is guaranteed. This pattern is very valuable and provides an effective analytical basis for evaluating different management approaches and their impact on the success of the organization. But this pattern is too general to have a direct impact on management decisions. Value index has been developed for this purpose. Value index is a simple ratio which determines the value of a management decision from the perspective of theory. The concept of value index is very clear, but its calculation requires real data and sometimes filed study. This index shows the extent to which a management decision about quality (and even non-quality) can make an organization more competitive. If this pattern and its mechanisms are continuously the foundation of management decisions for a long time, the organization obtains the ability to enter in heavy competition and can powerfully stand the threatening and unavoidable environmental phenomena. Perhaps if the managers of Pan American and Swiss Air had shaped their decisions by this logic during the last few decades, their brand would be still in flight.

#### **1.7. Sustainable (fixed) competitiveness advantage in e-commerce:**

Although competitiveness and achieving it is considered the key for success and superior performance of organization, it should be taken into account that competitive advantage must be sustainable. Obtaining a temporary competitive advantage cannot be effective for the organization and will be lost easily. In e-commerce, in which technological environment is considered a crucial factor and is rapidly and constantly progressing and changing, competitive advantage becomes more vital and it is more difficult to be maintained and sustained. Hence, organizations should seek to achieve a way that leads them towards gaining a sustainable competitive advantage. Amit, R. and Zott, C. (2000) described the way to gain competitive advantage through the internet as the main and vital tool in e-commerce. At the beginning, companies are in the same competitive state. In this state, the use of the Internet and its applications are almost identical in different companies. This continues until this balance is disturbed. This balance is disturbed when an organization uses the internet advantage at a

higher level. Internet must have the ability to generate income or reduce operating costs to be considered a valuable resource. If this occurs and the Internet generates revenue or reduces costs or both, the organization achieves a temporary competitive advantage. Internet should have some features such as scarcity, difficulty of imitation, and difficulty of replacement to be fixed as a valuable resource. If these conditions exist, organization achieves a minimum of temporary competitive advantage. However, it should be noted that organizations cannot maintain their competitive advantage by meeting these conditions in today's electronic and full of turbulence world, unless there be obstacles to the transfer of replacement and imitation of the source. Since the Internet is a global media, aforesaid obstacles cannot be realized. So, how the Internet can be considered the source of sustainable competitive advantage when it lacks these obstacles?

Studies show that competitive advantage is achieved when the Internet is applied as a supplement to other resources of an organization. The key point in e-commerce is that the Internet should not and cannot be used as a substitute for traditional tools of competition, but it should be used alongside traditional tools and as a supplement, because the Internet is not inherently a competitive advantage (Amit, R. and Zott, C. (2000)).

## 2. Discussions

Competitiveness can be defined as competencies and capabilities that a business, industry, region, and country possesses and can maintain them to produce a high return rate in manufacture parameters in the international competition and keep their human resources in a relatively high status. This have not possible without the participation of all employees. Recommendations system is the most effective system which is known to attract the participation of employees and other individuals associated with the organization. Correct implementation of this system is the first and most important step in achieving superior systems of quality and productivity. Competitive advantage must begin within the organization and then continue. For sustainable and profitable exports, environment and the market must firstly be identified, new markets should be created, and finally they must be attracted. There are different strategies to make an organization competitive. Deployment of competitiveness and excellence model in the organization is one of these mechanisms which leads to competition at the international level. A distinction strategy is valuable

when the customer is ready to pay much more money than production cost for its results in the product (good or service). In cost leadership strategy, this criterion emphasizes on the increase of product value (benefits-costs) from the perspective of the customer and the increase of marginal profit of manufacturer. These are the examples of the use of value-oriented attitude in improving the competitiveness of the organization. Nowadays, having a correct attitude to the concept of quality and understanding of its deep relationship with strategy and competitiveness are more important than the knowledge and skills of using the quality tools. We hope that the present paper is helpful in creation of this attitude.

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## Investigating the most important factors related to domestic violence rate towards children

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**Abstract:** The present study aimed at investigating the main factors related to parents' domestic violence toward children. This research conduct through survey method and the questionnaires was used to do this study in Ahwaz city. The population was all high school students in Ahwaz and their parents with sample size of 384 that calculated by Cochran formula. The sample was selected from various parts of Ahwaz city through cluster sampling and questionnaires were given to them randomly. To evaluate each of these variables, items were designed using a Likert or other scale and the required data was collected through questionnaire technique.

It is worthy to note that 55 questions of questioner related to independent variables including: social isolation and marital conflict statuses were answered by parents and 40 questions relates to dependent variable namely parental violence towards children were responded by students, in this study child abuse and trauma Questionnaire (CTQ) (Bernstein et al, 1995) the social isolation scale (UCLA) of Russell (1976) Marital Conflict Questionnaire (MCQ) Sanaei et al (2000) were used. The results indicated the significant correlation between marital conflicts and social isolation with parents' domestic violence toward children so it is worthy hat the families and the competent authorities pay more attention to the issue in order to prevent, control and decreasing its negative consequences at family and society level.

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**Keywords:** Domestic violence, social isolation, marital conflict

### 1. Introduction

Domestic violence is a global scourge and as nation's longstanding problem and no country or society is except from violence is not (Segal: 213 1999). Domestic violence is a problem which children are dealt with in all over the world and almost all children can experience Domestic violence to some extent and know it as a source of stress (Cummings, Korus and Pop, 2007). And its effects can be seen in other realms of social, educational, cognitive and psychological actions of children (Emery, Robert E.; Laumann-Billings, Lisa.1998). However, domestic violence against children seems a personal phenomenon at a glance, since its negative consequences disturb the order and health of society and it is the source of deviated behaviors such as misuse of narcotics and Alcohol, suicide, prostitution, escape from home, criminal behavior and sexual offenses, etc. necessitates the problem is considered as a very serious social damage. World Health Organization knows child maltreatment including all forms of physical or emotional abuse, sexual harassment, neglect, or negligent behavior, commercial or any other form of exploitation as a violence against children or child abuse which leads actual or potential harm child's health, survival, evolvment or dignity (Emiko A Tajima. 2000). According to the World Health Organization statistic (2005) forty million children are exposed to all kinds

of abuse in all over the world. Unicef also reported of killing 3500 children for physical violence and negligence, various statistics are estimated even in developed countries and the reality is more than formal statistics, any way, child abuse in any kind is unacceptable in any society is and considered a crime in many countries with legal aftermath (Brown,1998). Belsky, J findings (1993) shows that social loneliness and parental social restrictions lead to violence and neglect the children. JOHN FANTUZZO.et.al (1997) in their research findings showed that family with lower education, job statues, and income employment likely are more tensioned relations and domestic violence. Rennison, C. M., & Welchans, S. (2000) show that low illiteracy, poverty, unemployment, confidence, antisocial behavior, lack of social support and social loneliness of parents, their spousal abuse as well as substance abuse are considered as influential factors on child abuse Sullivan, C. M., & Bybee, D. I. (1999) indicated in their research that families with single parent, depressed parent, history of alcohol consumption by mother, parental violence exposure in childhood, and low household income experience significantly greater child abuse. Tajima, Emiko A, 2002 suggested that high family size, low education, parents' depression, social loneliness, lack of social support of parents, father's job and housewife mother are the other precipitating factors in child abuse.

Researches (Davies, P. T., & Cummings, 1994; Grych J., and Fincham, 1990) indicated the relationship between the child's exposure to parental violence leads to emotional problems (depression, anxiety) and behavioral (aggression and delinquency, suicide) in children. Parents' violence makes children stressed, fearful and angry - and repeated exposure of Conflict experience can lead to behavioral problems (Cummings and Davies ,1944) and Physical health problems in children (El-Sheikh, M, Harger, and Whitson, S,2001). Domestic violence is a potential threat for children and adolescent health and growth (chickti and Tooth, 1997). Parents' violence can influence children as a stress factor and longitudinal study predict high level of discord in 1 to 3 years old children after parents' conflict (Neyborz, forehead, and Bawo, 1997), (Katz, L.F., and Gottman, 1997).

Due to the patriarchal family system in Iranian society and tribal or traditional subcultures, poverty, unemployment, lack of proper parenting practices, poor education, lack of social support from family, rising divorce rates, increasing trend to drug, large size family, loss of family religion believe and numerous other factors, the domestic violence against children is increasing day by day. These factors led to the emergence of adverse outcomes at the individual, family and social level, such as aggression, anxiety, low self-esteem, delinquency, running away, suicide, drug abuse, and several other social injuries.

#### **1.1. Research objectives:**

1. Prevalence rate and kind of parental violence towards children
2. The relationship between social isolation and marital conflict and domestic violence towards children.

#### **1.2. Hypotheses**

- 1 – There is a significant relationship between the social isolation and parental violence towards children.
- 2 - There is a significant relationship between the marital conflict and parental violence towards children.

#### **1.3. Theoretical Research**

Since the parents' violence toward children is an effect of several causes and a theory explains just a specific aspect of it, a combination of theories of Blaksky's environmental theory (1980), Bandura's social learning theory (1978) has been used as the theoretical basis for this study.

#### **1.4. Environmental theory**

In "Blaksky environmental" theory, family external system or social formal and informal structures is considered important to explain family domestic violence matter. According to this theory, family's social loneliness and their social percept support from their external environment is affecting

factors on the incidence of violence against children (Blume, Thomas W. 1996).

#### **1.5. Social Learning Theory**

According to Bandura social learning theory (1978), behavior can be learnt through two ways: Either rewarding to actions (instrumental learning), or via deliberately or accidentally observation others' behavior (modeling). So the children, who somehow learn to be harsh, may continue the learned pattern till adulthood. Bandura also pointed to the underlying role of conditions such as socioeconomic status, population density, observing and experiencing parental violence in childhood and etc in the emergence of violence and aggression that can be used to explain the role of family aspect in child abuse (Boffey, Phillip M. 1983).

#### **1.6. Frustration -aggression theory**

According to Dollard frustration-aggression theory (1941), the frustration and failing is a result of stopping target-seeking behavior and is a cause of aggression. The violence may be directed to source of frustration or goals that are related to a primary source of frustration. The theory indicates the effect of frustration resulting from marital conflict on aggression toward children- as goals that relate to a primary source of failing

#### **1.7. Resource Theory**

According to William Goode source theory (1971), all the social systems (including family) have the authority system and anyone who access to major resources of family more than others can force the other members to obey his/her will. The mechanism of exerting authority is different based on the level of access of powerful person to other sources and the most powerful family member physical force resorts to exerting force via physical violence less than the others but the one with weak socioeconomic status, physical force and applying it is the only power source.

#### **2. Methods and variables measurement tools:**

This study is conducted via survey method using a questionnaire in Ahwaz city. The population is all high school students in Ahwaz and their parents in 2012; sample size was 384 calculated by Cochran formula. The number was selected by cluster sampling from various parts of Ahwaz and questionnaires randomly were given to them. To measure each variable, items were designed by Likert scale and the data was gathered through technique questionnaire (it is worthy to note that the 58 questions of questionnaire refer to Independent variables: socioeconomic status, social loneliness, marital conflicts that have been answered by the student's parents). In the research Childhood Trauma Questionnaire (CTQ) (Bernstein, 1995) is used to measure child abuse. CTQ is a self-report instrument

used to measure the level of injury in childhood. CTQ measure the abuse in five subscale of emotional abuse (EA), physical abuse (PA), sexual abuse (SA), Emotional Neglect (EN) and Physical Neglect (PN). Due to cultural issues, the item relate to sexual abuse was omitted. Generally, 40 items were applied to measure the level of parental violence toward children. In the study, the questionnaire and Children Trauma scale (CTQ) (Bernstein et al, 1995), Duncan (1986) socioeconomic statuses scale (SES), Russell (1976) social loneliness scale (UCLA), Sanaei et al (1379) Marital Conflict Questionnaire (MCQ) were used to measure variables. In this study, Duncan (1986) socioeconomic indicator was applied as the most widely used scale in the field of social research to measure the socioeconomic base, and three factor of job, income and education is assessed as a basis of social status. Marital Conflict Questionnaire (mcQ) is made based on Dr. B. Sanaei et al (2000) clinical experience to measure couples` conflicts. The questionnaire measures seven aspects of marital conflicts include decreased cooperation, decreased sexual relationship, increased emotional reactions, increased children support, increased personal relationship with her/his relatives, decreased familial relationship with his/her spouse relatives and friends, separating the fiscal affairs, in the study, the aspect of sexual relationship was omitted due to ethnic cultural sensitivity. Derived from Russell et al (1976) social loneliness (UCLA), in three aspects of familial, friendly, and neighborhood relationships were assessed in the form of Likert to measure the social loneliness. After respondents completed the questionnaire, data were analyzed using SPSS software at two levels of descriptive and inferential statistics. To measure the validity questionnaire, at first it was completed by subject, and then the validity coefficient was calculated by Cronbach's alpha to clarify items consistency.

**Table 1:** Cronbach's alpha coefficients for the variables and dimensions

Variable	number of items	statements Cronbach's alpha coefficient
Social isolation	18	0.88
Marital Conflict	37	0.79
Parental violence towards children	40	0.91

### 3. Research Findings

The findings show that the average and standard deviation for student age respectively are 16.01 and 0.98, for father`s age variable are respectively, 46.63 and 7.44, mother`s age variable, respectively, 40.21 and 5.98 as well for family size variable, they were respectively 5.56 and 1.67. Also, the findings suggest that in parents sample 38.5 % of subject were male and 61.5% of them were female. Also, in students

sample 34.1 percent of them were male and 65.9% of them were females.

### 1.3. Describing the variable of parents` violence toward children and different aspects of it

**Table 2:** Mean, standard deviation, minimum and maximum scores for the variables of parental violence towards children and its dimensions are listed.

Variable	items	Mean	Standard Deviation	Minimum	Maximum
Parental violence	40	64.80	17.56	44	141
Emotional violence	12	20.15	8.03	12	53
Physical violence	6	8.01	3.78	6	25
Emotional neglect	12	28.19	6.54	17	56
Physical neglect	7	8.44	2.74	7	27

As seen in the table above, the average and standard deviation were for variable of parental violence towards children, respectively, 64.80 and 17.56, for the emotional violence variables, respectively 20.15 and 8.0, for the physical variable respectively 8.01 and 3.78, for emotional neglect variables respectively 28.19 and 6.54 and for physical neglect variable respectively, 8.44 and 2.74

### 2.3. Testing hypotheses:

Hypothesis 1: there is a relationship between social isolation and parental violence toward children.

**Table 3:** Pearson correlation test results between social isolation and the extent of parental violence toward children

Independent variable	dependent variable		
	Number (n)	correlation coefficient (r)	significance level (p)
Social isolation	384	0.24	0.001
Relationships and socializing with relatives	384	0.29	0.001
Relationships and socializing with neighbors	384	0.24	0.001
Relationships and socializing with friends	384	0.26	0.001

As we can see in above table, the results of Pearson correlation test shows there is a positive significant correlation between parents` general social isolation and parental violence toward children ( $P=0.001$ ,  $r=0.24$ ). Also, there is a positive significant correlation between the dimension of socializing with family and parental violence toward children ( $r=0.29$  and  $p=0.001$ ). But there is a positive significant correlation between the dimension of socializing with neighbors and parental violence toward children ( $r=0.24$  and  $p=0.002$ ). There is a positive significant correlation between the dimension of socializing with friends and parental violence toward children ( $r=0.39$  and  $p=0.001$ ). Thus Hypothesis 1 is confirmed.

Hypothesis 2: there is a relationship between Marital Conflict and violence toward children.

**Table 4:** Pearson correlation test results between Marital Conflict and the extent of parental violence toward children

.Independent variable	dependent variable		
	Number (n)	correlation coefficient (r)	significance level (p)
Marital Conflict	384	0.293	0.001
decreased Partnership	384	0.275	0.001
Increased emotional reactivity	384	0.274	0.001
Attracting child support	384	0.245	0.001
Personal relationship with their relatives	384	0.05	0.25
Relationships with family and friends wife	384	0.313	0.001
Separate Fiscal affairs	384	0.06	0.18
Decreased relations	384	0.177	0.001

As can be seen in the table above, the Pearson correlation test result shows that there is a significant positive correlation between the total marital conflict and parental violence toward children ( $r=0.293$  and  $p=0.001$ ). As well there is a significant positive correlation between the decreased cooperation and parental violence toward children ( $r=0.275$  and  $p=0.001$ ). Also, there is a significant positive correlation between emotional reaction and parental violence toward children ( $r=0.274$  and  $p=0.001$ ). There is a significant positive correlation between

attracting child support and parental violence toward children ( $r=0.245$  and  $p=0.001$ ). There is not a significant positive correlation between the personal relationship with their relatives and parental violence toward children ( $r=0.05$  and  $p=0.25$ ). There is a significant positive correlation between Relationships with family and friends wife and parental violence toward children ( $r=0.313$  and  $p=0.001$ ). There is not a significant positive correlation between separating fiscal affairs and parental violence toward children ( $r=0.18$  and  $p=0.06$ ). There is a significant positive correlation between decreased relationship and parental violence toward children ( $r=0.177$  and  $p=0.001$ ). Thus, Hypothesis 2 is confirmed.

#### 4. Discussions

\* According to gained results it is clarified that the maximum and minimum variables of parental violence toward children are respectively emotional neglect, emotional coarseness, physical neglect, and physical coarseness. Also results the research shows there is a positive significant correlation between the levels of parents` general social isolation and their violence toward children, namely more social isolation more violence against children, it is consist with Glass (1972), Blesski (1993), Moss (1986).

\* Also, the research indicates that there is a positive significant correlation between the levels of parents` general marital conflicts and their violence toward children, namely more marital conflicts more violence against children, But there is not a positive significant correlation between the personal relationship with their relatives and separating fiscal issues and parents violence toward children. These findings are consistent with research findings from Berger (2005), William J., et al (2006), (Davies and Cummings, 1994; Grich and Fincham, 1990). Based on the results from multi variable regression, marital conflict have more effect than the other variables on dependent variable.

\*The overall research findings, this study could have an important achievement for practitioners, social planners of country mental along with formulating preventive primary and secondary plans against domestic violence against children and may not well pave the way for policy makers to task rules to decrease violence against children.

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**Analysis of Relationship between Brain Ischemia and Angiographic feature in Childhood Moyamoya Disease**ZHANG Zhiying<sup>1</sup>, WANG Li<sup>2</sup>, WU Jun<sup>1</sup>, XU Yuming<sup>3,\*</sup>

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**Abstract:** In 39 patients with childhood-onset moyamoya disease, angiograms were reviewed for stenocclusive lesions, and CT scans, MR images, or both were reviewed for the sites and extent of cerebral infarction. The relationship between the angiographic and CT/MR findings was examined. The prevalence and degree of stenocclusive lesions of the posterior cerebral artery (PCA) significantly correlated with the extent of lesions around the terminal portion of the internal carotid artery (ICA). The prevalence of infarction significantly correlated with the degree of stenocclusive changes of both the ICA and PCA. Infarctions tended to be distributed in the anterior borderzone in less-advanced cases. Our results indicate that progressive changes of the anterior and posterior circulations are associated with the distribution of cerebral infarction, culminating in a patchily disseminated infarction on CT and MR studies in late stages of the disease.

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**Keywords:** brain ischemia; cerebral angiography; moyamoya disease

## 1. Introduction

Moyamoya disease is a rare cerebrovascular occlusive disorder of unknown origin (1–4). It is divided into two types according to whether the onset occurs in childhood or adulthood (5). The main features of moyamoya disease are bilateral stenocclusive changes at and around the internal carotid artery (ICA) bifurcation along with a distribution of abnormal netlike vessels in the basal regions, called moyamoya (3). Although changes similar to those around the ICA can also be found in the posterior circulation, few reports have dealt with the posterior circulation in this disease (6, 7). In childhood-onset moyamoya disease, progression of cerebral infarction is considered to occur with an advancing stenocclusive process. However, no large-scale study has been undertaken to evaluate how the severity of stenocclusive vascular lesions in the anterior and posterior cerebral circulations is related to the development of cerebral infarction. Recent evidence indicates that the frequency of cerebral infarction positively correlates with the progression of posterior cerebral artery (PCA) lesions (7), but the relationship between the location and extent of infarction and the degree of stenocclusive PCA changes remains to be defined.

We studied the relationship between changes in the posterior and anterior circulations on angiograms and the frequency and extent of cerebral infarction on CT scans and MR images, or both, in 39 patients with childhood-onset moyamoya disease.

## 2 Methods

Between 1999 and 2011, 39 patients with children seen at our institution were confirmed to have moyamoya disease at angiography. 13 patients were male and 26 female; all patients were under 14 years of age (mean,  $8 \pm 3$  years) at the onset of symptoms. The average interval from the onset of symptoms to angiography was 5 years. None of the 39 patients had any other underlying disease, consistent with a diagnosis of idiopathic moyamoya disease. The initial manifestations of disease were transient ischemic attack or cerebral infarction in 36 patients; the other three patients presented with intraventricular hemorrhage at the age of 6 years, thalamic hemorrhage at the age of 6 years, and putaminal hemorrhage at the age of 10 years, respectively.

All 39 patients underwent cerebral angiography, including bilateral internal and external or common carotid arteriography, and unilateral or bilateral vertebral arteriography. All 39 patients were examined by CT ( $n = 39$ ), and 27 additionally underwent MR imaging. All CT and MR studies analyzed were performed within 1 month of cerebral angiography.

We applied two angiographic staging systems for the anterior and posterior circulations. We classified stenocclusive changes of the supraclinoid ICA into six angiographic stages as defined by Suzuki et al (3): stage I, narrowing of the carotid bifurcation only; stage II, dilatation of the main cerebral arteries with appearance of moyamoya vessels at or around the

terminal part of the ICA (ICA moyamoya); stage III, partial disappearance of the middle (MCA) and anterior (ACA) cerebral arteries with intensification of ICA moyamoya at the base of the brain; stage IV, advanced stenocclusive changes in the ICA (ACA and MCA are traced very dimly or in a completely different shape through the mist of the ICA moyamoya) with a small amount of ICA moyamoya; stage V, absence of the ACA and MCA with further reduction of the ICA moyamoya; and stage VI, blood supply only from the external carotid artery and almost complete disappearance of ICA moyamoya

The leptomeningeal collateral circulation frequently develops from the cortical branches of the PCA and from the posterior pericallosal arteries. The leptomeningeal collateral circulation from the PCA was subjectively classified into one of the following four grades according to its extent: good, cortical branches in all three frontal, parietal, and temporal lobes being more or less opacified; moderate, cortical branches in two of the three lobes opacified; poor, cortical branches in either the parietal or the temporal lobe opacified; none, no substantial collateral circulation.

CT and MR studies were reviewed to determine the location and number of cerebral infarctions. The number of infarctions was counted according to the regions involved, as described below. One continuous lesion involving two or more adjacent zones was regarded as two or more infarctions. Zones in the hemisphere were divided into the following eight regions: the territory of the ACA; the anterior half of the territory of the MCA (ant-MCA); the posterior half of the territory of the MCA (post-MCA); the territory of the PCA; the basal ganglia; and the thalamus. The ant-MCA and post-MCA were divided at the central sulcus, and the temporal lobe was included in the post-MCA.

Angiographic findings and CT and MR images were evaluated by two radiologists blinded to the patients' identity. CT and MR images were interpreted without knowledge of the angiographic findings. When interpretations were inconsistent, the final evaluation was reached by consensus. The interobserver agreement between the two radiologists was good: 94% in the interpretation of CT scans, MR images, or both, and 84% in the interpretation of angiograms.

The data were analyzed statistically by one of three methods: Spearman rank correlation, Mann-Whitney U-test, or Kruskal-Wallis rank test. Values of  $P < .05$  were considered statistically significant.

### 3 Results

Of the 39 patients, 22 (56%) were found to have stenocclusive lesions in one or both PCAs; 35 PCAs (45%) in 78 sides showed stenocclusive changes. The relationship between the ICA and the PCA stages of stenocclusive lesions is summarized in Table 1. The

degree of stenocclusive PCA changes significantly correlated with ICA stage (Spearman rank correlation,  $P < .0001$ ). Of the 19 sides with the most advanced stages involving the anterior circulation (ICA stages V or VI).

#### Leptomeningeal Collaterals from the PCA

Among the 78 sides, leptomeningeal collaterals from the PCA were seen in 42 sides (53%). These collaterals were scant in sides with ICA stage I and tended to be best developed in sides with ICA stage II or III. As the ICA stage advanced from III to VI, the degree of leptomeningeal collaterals from the PCA decreased significantly (Spearman rank correlation,  $P < .0001$ ). Of seven sides with ICA stage VI, representing the most advanced disease, only one (14%) had collaterals, which were very poorly developed. Also, there was a significant negative correlation between the PCA stage and the degree of leptomeningeal collaterals from the PCA (Spearman rank correlation,  $P < .0001$ ). (show in Fig 1).

#### Brain ischemia

Brain ischemia was demonstrated in 36 (46%) of 78 hemispheres. There was a significant positive correlation between the ICA staging and the number of infarcted regions (Spearman rank correlation,  $P < .0001$ ). Furthermore, except for the ACA territory, the more advanced the ICA stage, the more posterior regions were involved (Kruskal-Wallis rank test,  $P = .0005$ ).

The frequency of cerebral infarctions in the five regions other than the ACA territory significantly correlated with PCA stage. Furthermore, except for the ACA territory, the more advanced the PCA stage, the more posterior regions were involved (Kruskal-Wallis rank test,  $P < .0001$ ). Among the 9 sides with PCA stage 2, and the ant-MCA and post-MCA regions were more frequently involved than in PCA stage 1.

Table 1. Click on image to view larger version

ICA stage of stenocclusive lesions (side)	of	PCA stage of stenocclusion lesions			
		1	2	3	4
I	4	3	1	0	0
II	8	7	0	1	0
III	36	15	6	15	0
IV	13	4	3	5	1
V	4	1	0	1	2
VI	3	0	0	2	1
Total	78	30	10	24	4

Note number indicate the number of sides according to stage

Fig 1

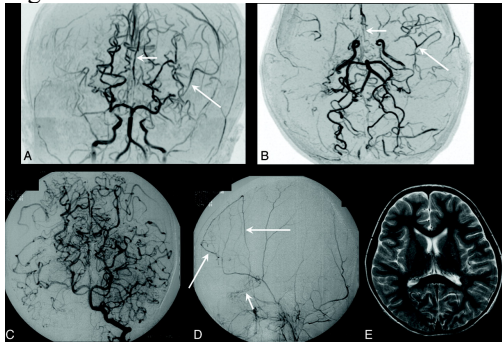


Fig 1 . A 7-year-old girl (diagnostic age group, 4–7 years) who had recurrent transient left hemiparesis for 5 years was diagnosed with Moyamoya disease, which manifested clinically as TIA. *A* and *B*, time-of-flight MR angiograms show advanced steno-occlusive changes at or around the terminal part of the right ICA, with poorly visualized ACA and MCA branches (ICA stage III, right). Moderate steno-occlusive changes at or around the terminal part of the left ICA with relatively good visualization of the ACA (*small arrows*) and MCA (*large arrows*) cortical branches (ICA stage II) are seen on the left. No steno-occlusive lesions are seen bilaterally in the posterior cerebral artery (PCA stage 1, bilaterally). *C*, Anteroposterior view of the vertebral angiogram shows no steno-occlusive lesions bilaterally in the PCA and well-developed leptomeningeal collateral circulation to the anterior circulation. *D*, Left lateral external carotid angiogram shows dilated anterior branches of the middle meningeal artery providing transdural collaterals to the contralateral frontal region (*large arrows*), with the medial branches of the maxillary artery providing transdural collaterals to the right anterior basal region (*small arrow*). Two transdural collaterals can be seen on the right, and none are seen on the left (anteroposterior view of the left external carotid angiogram, not shown). *E*, T2-weighted MR image shows no infarction bilaterally. Note that in this patient, the steno-occlusive changes do not involve the PCA, even in the right hemisphere, where ICA lesions are advanced (stage III).

#### 4 Discussion

Moyamoya disease is characterized by bilateral stenoocclusive changes at or around the terminal part of the ICA, with the development of abnormal netlike vessels, called moyamoya, at the base of the brain (3). Previous studies have focused on stenoocclusive changes of the anterior circulation, and PCA involvement is not included in the diagnostic criteria for this disease (8). Only a few studies have evaluated the posterior circulation (6, 7), even though the PCA is frequently affected in this disease. We found stenoocclusive changes of the PCAs in 58% of the 69

patients, with involvement of 62 (45%) of 137 hemispheres. The frequency is generally consistent with the findings of previous studies (6, 7).

In our series, the frequency of PCA involvement positively correlated with the ICA stage. This finding agrees with the recent work of Yamada et al (7), although the frequency of PCA involvement we observed in hemispheres with the most advanced ICA stages was quite different from theirs. ICA stages V and VI in our study correspond to ICA stage 5 in the classification used by Yamada et al. In hemispheres with such advanced ICA stages, the frequency of stenoocclusive PCA changes was 95% among our cases, in contrast to 59% in the study of Yamada et al. Although we have no obvious explanation for this large difference, it might have resulted from the difference in age of disease onset between the two studies. In our study, all patients were under 15 years of age (mean,  $6 \pm 3$  years) at onset, whereas the study of Yamada et al included patients with adult-onset disease, resulting in a mean onset age of  $10 \pm 12$  years; the difference in onset age between the two studies is statistically significant (Welch's t-test;  $P = .006$ ). We suspect, therefore, that vascular changes might progress faster in childhood (ie, the earlier the onset of disease, the faster PCA involvement may develop). Indeed, previous serial angiographic studies of patients with childhood-onset moyamoya disease have documented that the disease progresses up to adolescence but stabilizes or progresses very slowly after adulthood, and not all patients with moyamoya disease reach ICA stage V or VI (9).

We classified stenoocclusive changes of the PCA in accordance with the classification for the anterior circulation proposed by Suzuki et al (3). Their classification was based on the relationship between stenoocclusive changes of the main trunk and the intensification of and decrease in moyamoya vessels. In brief, collaterals, including moyamoya vessels, initially develop as occlusive changes of the ICA progress, and moyamoya vessels subsequently decrease when occlusive changes of the ICA become extremely severe. Such serial changes have been documented by follow-up angiograms (3). Although our PCA staging was not based on serial angiographic findings, the close relationship between ICA and PCA stages in our study supports that disease severity progresses in the posterior circulation in a similar manner to that in the anterior circulation. With progression of disease, occlusive changes are thought to occur initially in the proximal part of the PCA, with subsequent development of the PCA moyamoya (PCA stage 2) followed by gradual progression of stenoocclusive changes in the PCA and intensification of the PCA moyamoya (PCA stage 3). Finally, when the PCA is completely occluded, cortical branches are unopacified,

and the PCA moyamoya vessels decrease (PCA stage 4).

Cerebral infarctions in the MCA territory, the PCA territory were closely associated with stenooclusive changes in the PCA. Thus, when we see cerebral infarctions in the MCA territory, stenooclusive changes in the PCA are highly likely. Stenooclusive PCA changes are most probable, especially when the PCA territory is involved.

The positive correlation between the number of infarcted regions and ICA and PCA stage indicated a gradual extension of infarction in this disease. We postulate that the progression of stenooclusive PCA changes is closely related to such a pattern of development of cerebral infarction.

Kuroda et al (9) found that the occurrence of cerebral infarction did not correlate with stenooclusive ICA changes, but correlated with stenooclusive PCA changes, although stenooclusive ICA and PCA changes correlated with each other. In the present series, however, the frequency of cerebral infarction significantly correlated with the severity of both stenooclusive ICA and PCA changes. This discrepancy between the present study and that of Scott et al may reflect large difference in the frequency of PCA involvement in the hemispheres with the most advanced ICA stages, as noted above (95% vs 59%)(10).

#### Conclusion

We found that the severity of stenooclusive lesions in the PCA correlated positively with the severity of the stenooclusive ICA changes in childhood-onset moyamoya disease. Infarctions tended to be distributed in the anterior borderzone in the less advanced cases, while in the more advanced cases, lesions were additionally found posteriorly in the territory of the middle cerebral artery, the posterior borderzone, and the PCA territory.

10/2/2012

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## Determining and evaluating the requirements of ITS (Intelligent Transportation System) implementation in Iran road transport

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**Abstract:** Intelligent Transportation System (ITS) is a collection of amazing achievement of information technology in transportation which has transformed quality of people's lives as well as transportation management. Its use is essential given the increasing population and traffic increase. To take advantage of it is necessary to identify the main requirements of this system as its first architecture level given the area and location of its implementation and then the implementation conditions of next architecture levels is provided by determining the importance of each requirement and their components. This paper aims to determine and prioritize the requirements of architecture deployment of intelligent Transportation System in Iran and from the perspective of exports of this system in organizations in charge; thus, the statistical population is all export and specialists of ITS in seven organizations and companies involved and responsible in this field. For this purpose, 3 types of requirements have been introduced according to the research model: 1. the managerial structure; 2. Necessary contexts; 3. Technological requirements, and several indices have been proposed for each factor. The research method is descriptive-applied; and questionnaire and paired comparisons matrix have been used for collecting the required data, and SPSS software has been used for statistical analysis of information. The indices weights have been obtained using AHP method. In this study five research hypotheses have been proposed to investigate the existing and optimal conditions for deploying it; after the investigation, it was indicated that among the three introduced requirements in the model, the first and important priority in its implementation is managerial structure. It is worth mentioning that all three types of requirements are needed for implementing this project.

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**Keywords:** intelligent transportation system, system requirements, system architecture

### Introduction

Demand for travel is increasing every day, transportation becomes more widespread, and this leads to important issues such as congestion, increasing costs, accidents, wasting time and resources especially in roads and highways inside and around the cities [1]. Solving these issues is possible using this technology. Intelligent transportation system is a system that uses information and communication technologies to help handling the transport network. Tools of this intelligent system are used to avoid wasting time and sustaining lives of people besides improving the performance of the transport network [2]. These systems and services have been implemented worldwide. For example, navigation systems for vehicles are widely used in Japan while such system are not much common in the U.S.A tools of this system has three basic and central features including: information, communication, integration and cohesion that these three features help the transport executors to take more coordinated

decisions. Transport policies and measures are trying to adapt endless demands, more mobility with less traffic congestion, protect the environment and ensure safe and efficient operation of transportation system which requires more efforts in a wider area. One of these ideas is the use of intelligent transportation systems that can create a new horizon for achieving sustainable and safe mobility in the communication and transport network. Its advantages include the following: 1. Reducing accidents; 2. Helping to reduce traffic congestion; 3. Environmental monitoring and protecting; 4. Operating efficiency and productivity; 5. Comfort factors; 6. Increasing safety; 7. Customer satisfaction; 8. Saving energy (3).

Achieving these advantages is an important necessity in Iran; therefore, it is necessary to apply it and its basic requirements of its architecture must be determined. Determining the requirements in the architecture model of intelligent transportation system causes creating an analysis on the strengths

and weaknesses of facilities and existing resources in order to evaluate the existing opportunities for its deployment and provide a solution for dealing with threats and facilitate the codification of the strategy necessary to achieve the system objectives. To use it, first, basic requirements of its architecture must be determined and prioritized which has been discussed in this paper. In studies conducted in this field in Iran, the researcher only faced on sample by investigating numerous theses and articles, which only defined intelligent transportation system and its indices, while this article has been dealt with its implementation part in Iran road transport and how we can be benefited from its advantages in our country, and what factors must be noted more.

### Literature review

#### A: applying intelligent transportation system

Intelligent transportation system covers a wide range of new tools of administrating the transport network and servicing passengers. Tools of this system which are known as telemetric transport rest on three main features of information, communication and integration. Collecting, processing, aggregating and supplying information are its basis. In recent years, a number of countries have created these national plans and have designed and supported many of these projects. The experiences of these countries have been used for those who are ready to launch new plans and its projects.

Table 1: activities performed in the field of IT'S in selected and world's leading regions

location	year	activity	row
U.S	1967	Compilation of the first program of intelligent transportation system	1
U.S	1972	Implementation of intelligent control in five intersections	2
Europe	1985	Euro-K 4 transnational institution with the aim of developing IT in transportation	3
Europe	1991	Establishment of Ertico 5 transnational institution with the aim of compiling European standards and architecture of intelligent transportation system and determining Ertico goals for 2010	4
U.S	1992	Compiling architecture of intelligent transportation system	5
Japan	1995	Developing principles of intelligent transportation system	6
Japan	1996	Compilation of comprehensive plan of intelligent transportation system	7
U.S	1996	Compilation of twenty-year strategy of the intelligent transportation systems	8
Canada	2000	Comprehensive intelligent transportation system design and architecture	9
China	2001	intelligent transportation system design and architecture	10
Japan	from2004	Realization of the intelligent way	11

#### B: intelligent transportation architecture levels

In the past, traffic control systems were used to provide one or two services and these services were usually provided independently from each other; while multiple services that are provided to users simultaneously, there is a risk that a number of sub-components of the system does not work in opposition to each other. Intelligent transportation

At the national level, many countries have started this plan by establishing a collection of interested groups in public and private sector. Forming and maintaining this plan can greatly be facilitated by legislating or administrative orders at high governmental level [4].

Countries such as America, Canada, England, Australia, Japan and Netherlands are pioneering in the science and technology of transportation and traffic engineering, and have started initial studies on intelligent transport systems from 60s and 70s. in fact, these measures have been started after a few years of the need to perform management practices in the field of transportation. In these countries (and later in development countries such as south east Asia and smaller countries of Europe) the focus on infrastructural and hardware measure about traffic has come to an end long ago, and today the main emphasis is on performing projects and plans of system management improvement [5].

The important reference in evaluating this project for estimating costs and planning about implementing its programs is the U.S architecture documentation of intelligent transportation system which was presented in 1996 in order to provide the benefits than costs spent on its implementation. The activities performed in selected and world's leading regions have been presented in table 1:

architecture is needed to integrate system components and their cooperation and synergy which provide a framework for its design and implementation based on conditions and requirements.

As shown in the multilevel model of figure 1, its architecture is divided into different levels. It's essential that traffic and transport managers

concentrate their attention on the system architecture in levels order [6]; these levels are considered a range

for detailed engineering and design.

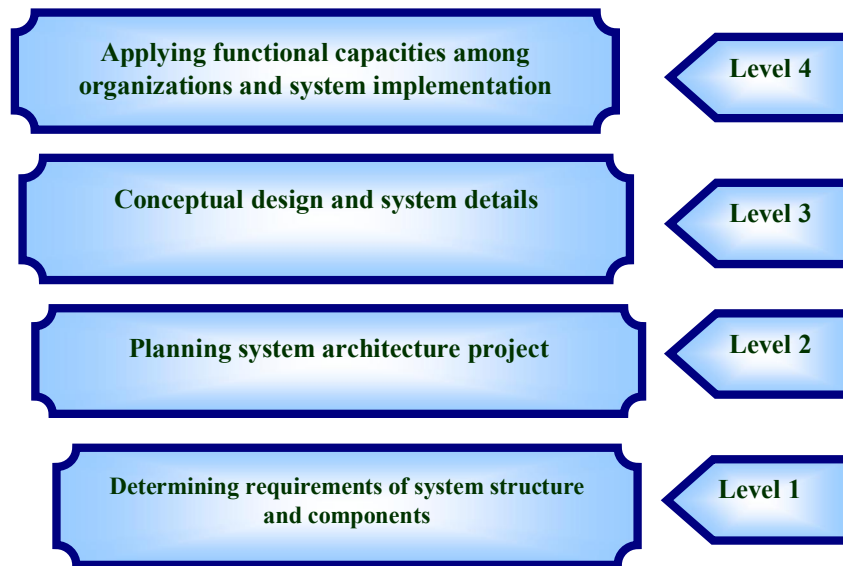


Figure 1: architecture levels of intelligent transportation [6]

According to researchers' opinions, it's essential that the first level of intelligent transportation architecture be started by determining and evaluating its criteria and indices from experts point of view [7].

#### C: the research model

To deploy and implement intelligent transport for using its advantages it's essential that main requirements of this system be identified as the first level of system architecture given the area and location of its implementation, and then the strategies for implementing next level of architecture be introduced given the importance of each requirement and their components. Figure 2 shows the research model including the requirements and their components in the deployment of this method.

#### Research hypotheses

Its implementation has caused new studies and consequently its development; its development has been concentrated in two areas of system management and tool and equipment [8].

The main issue of this study is to prioritize and determine the provided components in the analytical model and the two areas which will be implemented for roads and highways in Iran considering executive centers like Transportation Ministry, police and information centers. Given the condition of roads and traffic of highways in Iran and high waste of time and

volume of accidents and also the need to implement intelligent transportation system; thus, the first hypothesis is:

**Hypothesis 1.** In the existing and optimal situation, the most requirements in deploying intelligent transportation system are the factor of managerial structure.

Since management is an integral part of human life today and has had significant effects on various organizations and systems and enhanced the efficiency of using new technologies, accordingly the second hypothesis is:

**Hypothesis 2.** In the existing and optimal situation, the most important index of managerial structure indices is creating cooperation between executive units in the intelligent transportation system.

Today, countries using intelligent transportation system emphasize on creating the necessary conditions for optimal use of it. For example a country like Japan has established an independent telecommunication unit from public telecom for the optimum use of its services. According, the third hypothesis is as follows:

**Hypothesis 3:** in the existing and optimal situation, the most important index of necessary conditions is the condition of highways and roads in intelligent transportation system.

In developed countries massive investments are used for using intelligent transportation and the

latest technologies are used; and they have prevented accidents and wastes of time as much as possible, because which service has an efficiency and effectiveness compatible with its equipment. Accordingly, the fourth hypothesis is as follows:

**Hypothesis 4.** In the existing and optimal situation the most important index of technological requirements is receptors and stabilizers in intelligent transportation system.

Given that the four hypotheses above are considered from an overall view, it seems like asking you what

your idea about the football team is. You'll answer it's a good team with tactic, and all lines have plan. But another question is raised, what features must a forward have? This is the personal opinion which has been asked separately from organizations, accordingly, the fifth hypothesis is as follows:

**Hypothesis 5.** What is the priority of each requirements of intelligent transportation deployment in Iran roads for every organization?

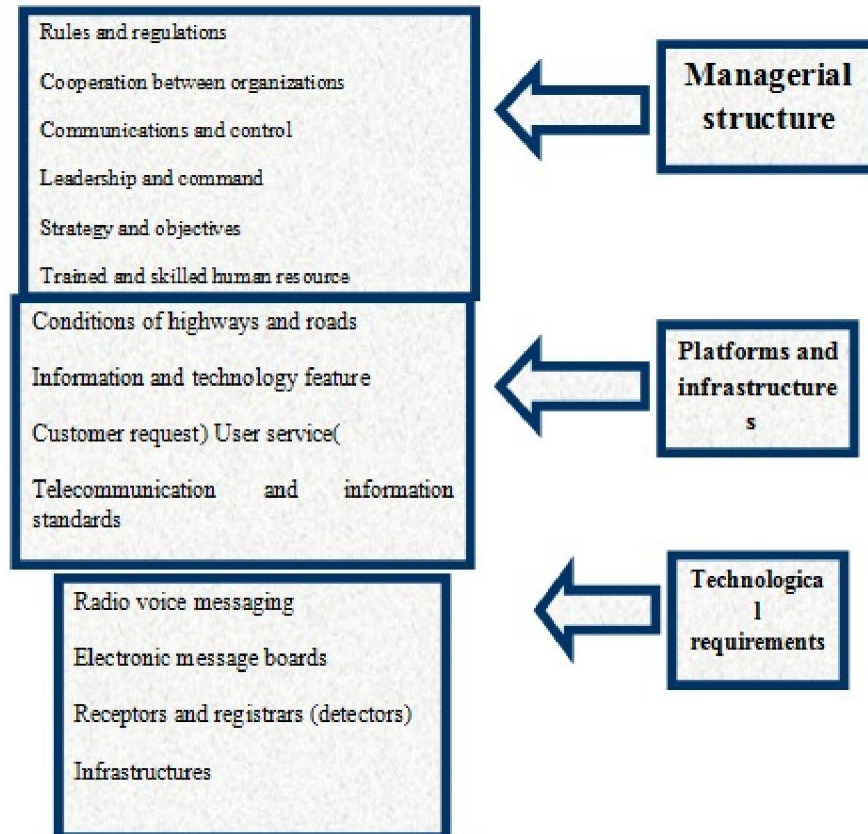


Figure 2. Analytical model of research, ITS implementation requirements [2] [9]

Answering the questions lead to achieve the research objectives as follows:

1. Determining implementation and deployment requirements of intelligent transportation system in Iran road transport
2. Determining and prioritizing deployment requirements of road intelligent transportation system for changed organizations

### Research methodology

This is a descriptive-applied study. Descriptive method has been used for describing its factors and indices in the statistical population; and the applied method has been selected since the

research objective is applying the results in the statistical population.

The statistical population of this study is all organizations and companies involved and responsible in its field. The number of organizations and companies is seven which has been listed in table 1. All related experts and specialists are considered as statistical samples. Detecting experts and specialists of this system has been performed in by person visiting and the study by the researcher in desired organizations and companies; in each organization, available experts and specialists have been identified through organizational chart and the opinion of the organization managers; and after initial interview the researcher has found that they have been skilled in



the field of transportation and traffic in Iran besides having the knowledge of this system. Details and their number have been provided in table 2.

Since this is an attitude survey study, the researcher based his work on questionnaire technique which has been used for collecting the required data. In order to identify important factors affecting the implementation of this system, by studying authentic and related books and articles and also obtaining the views of experts, the questionnaire was made with closed questions with five options (very much (5), much (4), medium (3), low (2), very low (1)) by the researcher. The five-opinion questionnaire was designed for evaluating indices of this system implementation in the existing and optimal situation in Iran using the perspective of selected organizations and companies. Also, questionnaire 2 was prepared and arranged for performing paired comparisons and prioritizing influential factors from specialized perspective for each organization and company. To determine the validity of questionnaires along time was spent on interviewing and contacting with the experts in the statistical population.

Table 2: statistics of expert/specialist in intelligent transportation in organizations and companies of statistical population

Number of specialist/expert of intelligent transportation	Name of company/organization
12	Road maintenance organization
10	Transport institute
14	police
10	Traffic control
4	Arman Company
3	Metra Cmpany
3	Tehran Arg Company
56	Total

After ensuring the validity, the final questionnaire was given to 10 employees of statistical population and completed in order to determine its reliability. Then, its reliability was calculated by Cronbach's alpha method using SPSS software. Its results are summarized in table 3.

Table 3: the value of Cronbach's alpha for each of key factors using SPSS software

Chronbch's Alpha	Main factors	Number of factors
0.85	Managerial structure factor	1
0.89	Platforms and infrastructures factor	2
0.88	Technological requirements factor	3

Since the obtained coefficients are higher 75%, the reliability of the questionnaire is acceptable.

#### A: the mode of information analysis stages of the research

In order to simply introduce how information has been analyzed, the model of information analysis stages of the research has been presented in figure 2.

To determine implementation requirements of this system in road traffic, data were evaluated in the two required conditions (ideal) and existing condition. To this end, a questionnaire was designed qualitatively and prepared for analysis by ranking the options, 1 for very low to 5 for very much.

The statistical sample has been divided into 5 groups including road maintenance organization, companies, traffic control organization, transport research organization and the police. The questionnaire was distributed among those who had a complete or partial recognition to intelligent transport and its performance. 12 questionnaires were delivered for the road maintenance organization, 7 for companies, 6 for transport institute, 7 for Tehran control traffic organization and finally for police authorities. By testing the mean difference between groups, it was indicated that there was no significant difference between them. Thus, the groups were converted to one sample for ease in reviewing the statistical questions.

#### B: statistical analysis

Each of the questions 1 to 4 was tested for the situation in Iran using descriptive statistic method. Question 5 has been evaluated using AHP method. Question 5 is to determine the priority of requirements from the perspective of decision makers of each organization for that organization; actually it is ranking indices relative to each other that AHP method is suited for this purpose. This method wants the decision maker to compare indices with each other through paired comparisons matrix; and the final W vector in which the weight of each index is from 0 to 1 is obtained with a simple averaging process. In this method, a natural measure of adjustment degree of the decision maker comparison is calculated [10].

#### Results

1. B: evaluating the first question

A. 1-B: evaluating the first question in optimal situation

To test the first hypothesis, the calculated mean of optimal or required situation of factors for implementing this system is desired. The acceptable mean number is 3. Now considering that groups'

mean is larger than the acceptable mean, therefore, the factor of managerial structure is a requirement for its implementation. Table 4 lists a summary of evaluating factors affecting implementation of intelligent transportation.

Table 4: the results of evaluating the first question is the optimal situation

Factor rank	Optimal mean	Acceptance mean	factors
1	4.71	3	The first factor of managerial structure
3	4.29	3	The second factor of conditions and infrastructures
2	4.45	3	The second factor of technological requirements

By comparing factors' means, it was indicated that managerial structure factor with the mean of 4.71 is the most important factor, next technological requirements factor with the mean of 4.45 and the third one infrastructures factor with the mean of 4.29 for the implementation of intelligent transportation.

**B. 1-B: evaluating the first question, comparing the existing situation with optimal**

The evaluation of the first question has been performed for each factor by comparing the existing situation with optimal. The obtained difference represents the most requirements for that factor.

Evaluating the mean of the first factor in the existing and optimal situation.

Table 5: the first factor mean in the existing and optimal situation

mean	Acceptance mean	
1.74	3	<b>Existing situation</b>
4.71	3	<b>Optimal situation</b>

To deploy this system, the first factor must be at least as much as 4.71 from 5 while in the existing situation it is less than the average of 1.74 from 5. Evaluating the second factor mean in the existing and optimal situation

Table 6: the second factor mean in the existing and optimal situation

mean	Acceptance mean	
2.61	3	<b>Existing situation</b>
4.29	3	<b>Optimal situation</b>

To deploy this system, the second factor must exist at least as much as 4.29 while it is less than the average of 2.61 in the existing situation. Evaluating the third factor mean in the existing and optimal situation.

Table 7: third factor mean in the existing and optimal situation

mean	Acceptance mean	
2.11	<3	<b>Existing situation</b>
4.45	>3	<b>Optimal situation</b>

To deploy this system, the third factor must be as much as 4.45 while it is less than the average of 2.11 in the existing situation. Diagram 1 shows the results of evaluating the three factors simply.

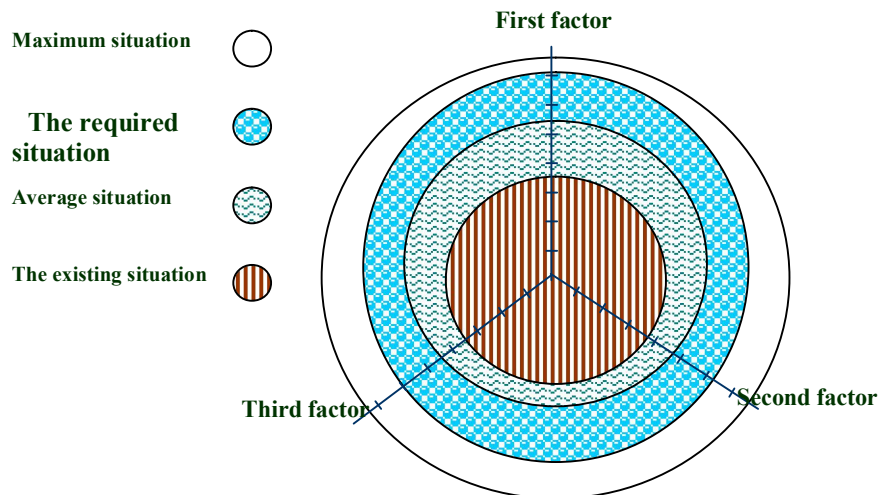


Diagram 1. The existing and optimal situation of the triple factors in the implementation of intelligent transportation

The result is that in terms of statistical population in the existing situation, none of the factors have the required conditions for implementing this system.

### C – B: evaluating the second question

The most important requirement of managerial structure indices is the index of creating cooperation between executive units in intelligent transportation system.

A – the evaluation of the second question in optimal and existing situation and comparing the existing situation with optimal one for each index of the first

factor have been done using SPSS software and listed in table 7.

The most important index in its implementation is an index which requires the most need in the necessary conditions and has the worst condition in the existing situation; because the research objective is attracting those who interested in the weakest indices in the current condition of Iran transportation which require the most attention. For an overall conclusion the distance of the existing situation with the required situation (optimal) is considered and the most distance suggests the most important index which must be taken into consideration. Table has been prepared to this end.

Table 8: determining the distance between the existing and optimal situation in the first factor indices

Attention priority	distance	rank	Existing situation	rank	Optimal situation	index
4	2/38	2	2/42	2	4/8	Rules and regulations
2	2/47	6	1/93	6	4/4	Communications and control
1	2/6	5	2/2	1	4/8	Creating cooperation between organizations
3	2/44	4	2/21	5	4/65	Leading and commanding
5	2/3	3	2/4	4	4/7	Strategy and objectives
6	2/28	1	2/52	3	4/8	Trained human resource

Based on the information in table 8 in optimal situation, the most important index of the managerial structure is creating cooperation among organizations. The most distance to reach the optimal situation is the third index, i.e. creating cooperation between units in the managerial structure factor.

### D –B: evaluating the third question

What is the most important index of the second factor, i.e. platforms and infrastructures in intelligent transportation system?

Descriptive statistics of the third questions in the existing situation has been presented in table 9.

Table 9: the distance between the existing and optimal situation in the second factor indices

Attention priority	distance	rank	Existing situation	rank	Optimal situation	index
3	1.37	3	2.62	2	3.99	First index
1	2.38	4	2	3	4.38	Second index
4	1.115	1	3.2	4	4.315	Third index
2	1.96	2	2.7	1	4.66	Fourth index

Among the second factor indices, providing financial condition and compiling standards have the most requirements.

### E – B: evaluating the fourth question

What is the most important requirement of technological requirement in intelligent transportation system?

Table 10: the distance between the existing and optimal situation in the third factor indices

rank	distance	rank	Existing situation	rank	Optimal situation	index
4	1.25	1	3	4	4.25	First index
3	2.15	2	2.4	3	4.55	Second index
1	3.05	4	1.7	1	4.75	Third index
2	2.8	3	1.8	2	4.6	Fourth index

Therefore, the most important index of technological requirements which must be taken into

consideration is the index of receptors and registrars in intelligent transportation.

**C: determining the indices weights from the specialized perspective of each organization**

In designing this system besides identifying the impact of factors, the priorities of the involved and responsible organizations for the implementation of intelligent transportation must also be determined; so that targeting is done with a comprehensive view to provide the compatibility of units and their synergy besides their integrity. They also must prioritize executive indices based on their own requirements and use them as primary planning inputs [11]. Moreover, the required cooperation and integrity does not exist between related organization and their status in the system is not specified; and private sector must be used for designing this system [12]. For this reason, a survey was performed from specialists of each organization using paired comparisons matrix and the weights of indices have been determined with AHP method. Because of the lengthy calculations, the calculation process for the first factor indices of the Road Maintenance

Organization has been presented; and for other factors and organizations, only results have been listed in table 14.

Determining weights of the first factor indices from the perspective of Iran Road Maintenance Organization. First, the geometric mean of the corresponding entries to the paired comparisons matrices has been obtained for factor indices in table 11.

Table 11: the geometric mean of paired comparisons matrices for the first factor indices

	1	2	3	4	5	6
1	1	1.54	1.46	1.34	1.7	1.87
2	0.67	1	1.52	1.58	2.09	2.09
3	0.66	0.65	1	2.07	2.008	1.86
4	0.74	0.64	0.56	1	1.61	1.4
5	0.58	0.47	0.49	0.61	1	1.2
6	0.55	0.47	0.53	0.7	0.58	1

Then weight of indices was obtained using geometric mean.

$$\begin{array}{c}
 \xrightarrow{\text{Lines Geometric Average}} \begin{array}{|c|} \hline 1.43 \\ \hline 1.38 \\ \hline 1.22 \\ \hline 0.91 \\ \hline 0.97 \\ \hline 0.61 \\ \hline \end{array} \xrightarrow{\text{Normalization}} \begin{array}{|c|} \hline 0.23 \\ \hline 0.22 \\ \hline 0.19 \\ \hline 0.14 \\ \hline 0.12 \\ \hline 0.10 \\ \hline \end{array} = \omega
 \end{array}$$

Now, the priorities obtained from judgments of group members must be trusted. To this end, the consistency rate has been obtained for the comparisons. The studies have shown that if consistency rate (CR) is less than 10 percent, the

consistency of comparisons can be accepted [10], otherwise, the comparisons should be done again. The consistency rate has been calculated through a special vector method as follows:

$$A\omega = \lambda\omega \Rightarrow \begin{array}{|c|} \hline 1.39 \\ \hline 1.34 \\ \hline 1.20 \\ \hline 0.88 \\ \hline 0.65 \\ \hline 0.59 \\ \hline \end{array} = \lambda \begin{array}{|c|} \hline 0.23 \\ \hline 0.22 \\ \hline 0.19 \\ \hline 0.14 \\ \hline 0.12 \\ \hline 0.10 \\ \hline \end{array} \Rightarrow \begin{array}{l} \lambda_1 = 6.04 \\ \lambda_2 = 6.09 \\ \lambda_3 = 6.31 \\ \lambda_4 = 6.2 \\ \lambda_5 = 5.90 \\ \lambda_6 = 6.5 \end{array} \rightarrow \bar{\lambda} = 6.18$$

$$IR = \frac{II}{IIR}$$

$$\lambda = \frac{\sum_j a_{ij} w_j}{w_i} \quad II = \frac{\bar{\lambda} - n}{n - 1} = \frac{6.18 - 6}{5} = 0.036$$

IIR is a random index which has been calculated in resources for n from 1 to ...; the number in this study is 6 so IIR=1.24.

<i>n</i>	2	3	4	5	6	7	8	9
<i>IIR</i>	0	0.58	0.9	1.12	1.24	1.32	1.41	1.45

$$IR = \frac{0.036}{24.1} = 0.02 \quad IR = \frac{II}{IIR}$$

Therefore, with 0.02 consistency rate in the judgment of specialists, thus, the priority of indices for Iran Road Maintenance Organization is as follows:

1. Determining strategies and objectives
2. Creating rules and regulations
3. Cooperation among the necessary organizations
4. Communication and control
5. Determining leadership and command

Table 12 shows weights of intelligent transportation indices in every organization or company.

Table 12: weights of intelligent transportation system in each organization or country

Results and priorities			Name of organization/company	row
Requirements factor	Platforms factor	Managerial structure		
-message radio -message boards -detectors -Telecom infrastructures	-infrastructures -information feature -Customer request -standards	-strategy and objectives -rules and regulations -cooperation among organizations -communication and control -leadership and command -Human resource	Road maintenance organization	1
- message radio -message boards -detectors -Telecom infrastructures	-infrastructures -information feature -Customer request -standards	-strategy and objectives -rules and regulations -cooperation among organizations -communication and control -leadership and command -Human resource	Transport institute	2
-detectors - message radio -Telecom infrastructures -message boards	-infrastructures -information feature -Customer request -standards	-strategy and objectives -rules and regulations -cooperation among organizations -communication and control -leadership and command -Human resource	Traffic control organization	3
-detectors -Telecom infrastructures - -message boards - message radio	-infrastructures -information feature -Customer request -standards	-strategy and objectives -rules and regulations -cooperation among organizations -communication and control -leadership and command -Human resource	Tehran Arg company	4
-detectors -Telecom infrastructures - -message boards - message radio	-infrastructures -information feature -Customer request -standards	-strategy and objectives -rules and regulations -cooperation among organizations -communication and control -leadership and commanding -Human resource	Metra consultation company	5
-message radio -message boards -detectors -Telecom infrastructures	-infrastructures -information feature -Customer request -standards	-strategy and objectives -rules and regulations -cooperation among organizations -communication and control -leadership and commanding -Human resource	The police	6
-detectors -Telecom infrastructures - -message boards - message radio	-infrastructures -information feature -Customer request -standards	-strategy and objectives -rules and regulations -cooperation among organizations -communication and control -leadership and commanding -Human resource	<b>Arman Company</b>	7

## Discussion and conclusion

Given that intelligent transportation is a technology that its implementation depends on environmental conditions and facilities; thus, in connection with its implementation, the opinions of in charge and executive organizations must be considered; and regarding the overall summary of the views of organizations involved in this system, cases and indices are considered for its implementation [13], this research has been conducted with this view and the following results have been presented.

In the present study, four questions have been considered for evaluating factors and indices in the existing and optimal situation for its deployment in Iran; and AHP has been used for determining the importance and weights of important indices in the deployment of this system from the perspective of each organization. To select the most important among the three factors affecting its architecture in the situation in Iran by comparing the existing and optimal situation, it was indicated that managerial structure factor is more important than physical requirements and platforms, i.e. among the three proposed factors, the factor of managerial structure must be taken into consideration more than other factors. Therefore, by using efficient methods between involved and in charge organizations for its implementation, the required connection must be created to be able to coordinate other activities for implementing among organizations. Because by implementing this factor, other factors can be implemented as well. It should be noted that for the implementation of this system, all three factors in the provided model have been known as requirements of its implementation.

The purpose of second, third and fourth questions is identifying the most important indices of factors; the result is

1. Managerial structure factor as the most important factor for intelligent transportation is the index of creating cooperation and coordination between involved units in its implementation such as police, road maintenance, etc.
2. The most important index in the second factor of this system has been the compilation of standards, and this result seems logical after an analytical evaluation, because the base of platforms is the presence of executive standards, and receptors and registrars must be designed and deployed considering these standards.
3. The most important index of the third factor, i.e. technological requirements of intelligent transportation is receptors and registrars.

The priority and importance of indices for each organization have been determined using AHP method which has been presented in table 12. According to results of this table in the dimension of managerial structure among the proposed indices for all organizations, the most attention must be paid to the index of creating cooperation between groups and organizations involved in intelligent transportation; thus for creating the ground for its implementation, the compilation of telecommunication and information standards must be considered, because other indices in the factor of grounds are related to this index.

Because in the factor of physical requirements, the index of deploying electronic message boards has been more important. Therefore, the ground of the required planning and budgeting must be done for preparing the appropriate equipment.

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## Effect of hyperoxygenation for one minute on ABGs during endotracheal suctioning in ICU in Zanjan Vali-e-Asr hospital 2011

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**Abstract:** Background and objective: Endotracheal suctioning is an inevitable procedure in patient under mechanical ventilation. The most important complication of this procedure is hypoxemia. The aim of this study is effect of hyperoxygenation on hypoxemia during endotracheal suctioning. Method: This study is a clinical trial on 30 patients under mechanical ventilation in ICU of Vali-e-Asr hospital in Zanjan that samples are selected based on study criteria. Results: Finding show that hyperoxygenation during endotracheal suctioning is necessary. Based on results of this study recommended this procedure and further study for distinct during of time of hyperoxygenation.

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**Key words:** Hyperoxygenation, Endotracheal suctioning

### Introduction

Using artificial airways such as intra-tracheal tube is inevitable in patients that undergo mechanical ventilation with positive pressure due to different causes. By placing the tube within the trachea and the patient's inability to cough and removal of pulmonary secretions, which may be added as a considerable amount due to the disease process, performing the pulmonary secretions removal technique with suction help would be needed. Endotracheal tube suctioning is a stressful and uncomfortable process for the patients; however, it is essential for clearing the tracheobronchial tree from the pulmonary secretions. Suctioning has several side effects on different body systems, including:

1. Respiratory system (Lung volume reduction, hypoxia, alveolar collapse and trachea infection and trauma)
2. Circulation system (Bradycardia and hypotension)
3. Nervous system (Increased intracranial pressure and decreased cerebral blood flow)

Despite observing such complications, there is little evidence and conducted research on incidence and prevalence of these side effects and performing preventive measures in this regard (American Association of Respiratory Care, 2009, AARC).

There are several different systems for tracheal suctioning, including: open suctioning system, semi-open suctioning system and closed suctioning system.

In open endotracheal suctioning system, which is now used in Hazrat-e-Valiasr Hospital, the tracheal tube is removed at the place of Y piece from the ventilator and the suction catheter will be entered

into the endotracheal tube. In this method, disconnecting the patient from the ventilator causes the reduced airways pressure equal to the atmospheric pressure. In the second method, which is called the semi-closed method, without disconnecting the patient from the ventilator, the suction catheter removes the patient's respiratory secretions through a lateral hole created by the help of an adaptor at the junction site of ventilator to the endotracheal tube. In this method, pressure reduction, and thus, the reduced volume of the lungs will be avoided partially. But in the final approach, which is called as the closed method, the suction catheter is continuously placed between the endotracheal tube and the Y piece, and without disconnecting the patient from the ventilator, the secretions are suctioned (Al-Megren, 2005).

One of the important complications of endotracheal suctioning that other side effects may be due to the same problem includes the hypoxia induced by disconnecting the patient from the ventilator and applying negative pressure to remove the secretions. To reduce the hypoxia, methods such as hyperinflation and hyperventilation are used. One of the measures done to reduce the hypoxia induced by endotracheal suctioning in mechanically ventilated patients is hyper-oxygenation before and after suctioning of secretions. In this method, before and after endotracheal suctioning, the patient is given pure oxygen (100% oxygen). Hyper-oxygenation may be performed through equipments that are usually mounted on the ventilator or manually after disconnecting the patient from the ventilator (Demir, 2004).

Using high percentage of oxygen during endotracheal tube suctioning is not without risk for the patients. It has been proven that high percentage

of oxygen for even a short time can cause absorptive atelectasis in normal subjects. In patients with lung problems treated by high percentage oxygenation, the caused atelectasis and subsequent lung volume reduction will worsen the patient's condition. Thus, unnecessary use of pure oxygen for patients is dangerous. Control of oxygen saturation of arterial blood ( $O_2\text{sat}$ ) before and after endotracheal tube suctioning is necessary, and if the oxygen saturation rate of arterial blood reduces, treating the patients with high percentage oxygen considering its complications will be essential.

Most ventilators have the opportunity to provide 100% oxygen at a predetermined time that will cause minimum hemodynamic side effects for the patient (Thompson, 2000).

By reviewing the researches conducted on hyper-oxygenation, arterial blood oxygen saturation and other parameters included in the study of arterial blood gases, it was found that no specified and constant time has been recommended for performing hyper-oxygenation before and after endotracheal tube suctioning. Thus, in order to determine the effect of hyper-oxygenation period time before and after endotracheal tube suctioning and compare the influence of different period times on the arterial blood oxygenation rate, and ultimately help in determining the appropriate time for hyper-oxygenation, the researcher intends to examine the effect of different period times of 100% oxygen administration on the parameters included in the study of arterial blood gases in a clinical trial in this study.

### Materials and Methods

The current study is a clinical trial that was performed aimed at study of hyper-oxygenation period time influence on the study parameters of arterial blood gases (ABGs) in patients under mechanical ventilation in ICU ward of Hazrat-e-Valiasr hospital. The study samples were 30 patients, which were calculated using the Cochran's sample size formula. The samples had the following characteristics: age over 18 years old, being mechanically ventilated for more than 48 hours, having mild to moderate respiratory failure, lack of hyperthermia, hematocrit rate greater than 25, sustained hemodynamic status and 7.5-8 endotracheal tube.

Data collection tool was a recording data sheet, which is composed of two parts. In the first section, the demographic characteristics of the samples were collected and were recorded based on Demir, F. research factors including, sex, age, diagnosis, the airway type and the ventilation type. In the second section, the data were related to the measurement of arterial blood gases parameters,

including  $\text{PaO}_2$ ,  $\text{PaCO}_2$ , PH and  $O_2\text{sat}$  that was performed using the arterial blood gases study device by Techno media 630 IE model.

The content validity method has been used to determine the validity of the method of data collection (by 11 faculty members). The simultaneous observing technique was also used to determine the reliability. The reliability of the arterial blood gases analysis system was evaluated based on the manufacturer's instructions.

The researchers selected the samples and performed the sampling according to their skills in ICU section and with the assistance of a qualified physician (for verification and identification of research samples and interpretation of data in ABGs). After taking the initial ABGs sample and its recording, in the case of endotracheal tube secretions and the need for its suctioning, the suctioning of the endotracheal tube secretions was performed by doing hyper-oxygenation with 100% oxygen for one minute before and after the suctioning.

The ABGs results were interpreted and compared by the physician partner. Then, the data was entered into the SPSS software. The statistical analysis was performed by two descriptive and inferential methods (hypothesis testing, comparison of two dependent samples).

The study exclusion criteria included the use of muscular paralyzing drugs and bronchodilator and changes in ventilator settings during performing the research. Written consent was taken from the conscious patients prior to performing the research, and in unconscious patients, the written consent was obtained from their relatives. Sampling was performed from the femoral artery.

### Results

To achieve the research objectives, data was collected and examined using descriptive and inferential statistics as follows. First, to describe the studied variables, the means of arterial blood gases study parameters ( $\text{PaO}_2$ ,  $\text{PaCO}_2$ , PH and  $O_2\text{sat}$ ) were measured before doing the suctioning of tracheal tube secretions and after hyper-oxygenation for one minute and were recorded in Table 1.

Table 1: The means of study parameters of arterial blood gases of initial ABGs and ABGs after one - minute hyper-oxygenation and the tracheal tube suctioning

Variables	$O_2\text{sat}$	$\text{PaO}_2$	$\text{PaCO}_2$	PH
ABGs turn				
ABGs <sub>0</sub> *	92.73	90.28	38.88	7.37
ABGs <sub>1</sub> *	95.56	89.94	45.91	7.27

ABGs<sub>0</sub> \*: ABGs before endotracheal tube suctioning  
 ABGs<sub>1</sub> \*: ABGs 30 seconds after performing hyper-oxygenation for one minute.



After recording the means, means comparison was performed that the following results were obtained:

About the O<sub>2</sub>sat parameters, with P=0.015, PaCO<sub>2</sub> with P=0.00 and PH with P0.006, there was a significant difference between the two samples. But in case of PaO<sub>2</sub> parameter with P=0.92, no significant difference was observed between the two means.

### Discussion

Regarding the measurement of study parameters of arterial blood gases immediately before performing tracheal tube suctioning, the means of parameters were measured. The results show that the research samples have no acute respiratory problems or there are no drastic changes in the values of PaO<sub>2</sub>, PaCO<sub>2</sub>, PH and O<sub>2</sub>sat.

In this regard, hyper-oxygenation for one minute and the endotracheal tube suctioning of the patient were performed, and the ABGs were performed 30 seconds after suctioning.

By examining each of the studied variables and comparing the means of variables, it was seen that the difference between variables is significant. Then, the result of this study shows that using hyper-oxygenation during tracheal tube suctioning not only prevents the hypoxia induced by tracheal tube suctioning, but also leads to improvement of the initial situation of the patient O<sub>2</sub>sat. Prior to this, researchers such as Xu-Yang-Ping (2004) has found the effect of endotracheal suctioning on the patient O<sub>2</sub>sat, and in a research conducted by Wang et al., they recommended hyper-oxygenation in order to prevent hypoxia induced by suctioning the endotracheal tube secretions (Wang 2005).

In a research conducted by Abbas Ebadi in Baqiyatallah Jamaran Hospital, he studied the effects of hyper-oxygenation, hyper-inflation and hyper-ventilation on hypoxia induced by suctioning and concluded that the hyper-oxygenation not only prevents the hypoxia, but also it certainly is preferable to the other methods (Ebadi, 2009).

Searching of the literature, we found that although the researcher have found the importance of hyper-oxygenation in reduction or prevention of hypoxia-induced following endotracheal tube suctioning and will recommend it, but they have not determined a specific time period for it. For example, Fernandez used a period time of two minutes for hyper-oxygenation in his study (Fernandez, 2004), and Demir selected the time of one minute to perform this procedure.

The time period of hyper-oxygenation has not been also specified in Ebadi's research. It should

be noted that by review of conducted studies with an emphasis on performed hyper-oxygenation before and after endotracheal tube suctioning, the definitive effect of hyper-oxygenation on study parameters of arterial blood gases, particularly PH and PaCO<sub>2</sub> has not been already determined, and no source was found to measure the effect of hyper-oxygenation on PH and PaCO<sub>2</sub>.

### Final result

In patients undergoing mechanical ventilation, performing hyper-oxygenation for one minute during tracheal tube secretions suctioning leads to improvement of hypoxia and prevention of the hypoxia caused by this procedure. Although performing hyper-oxygenation increases O<sub>2</sub>sat, but it will not change the amount of PaO<sub>2</sub> significantly, and despite of hyper-oxygenation, performing tracheal tube suctioning of secretions will lead to increased PaCO<sub>2</sub> and reduced PH.

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