Available online at www.jpsscientificpublications.com
Volume – 2; Issue - 6; Year – 2016; Page: 843 – 855
DOI: 10.21276.iajmr.2016.2.6.2

Indo – Asian Journal of Multidisciplinary Research (IAJMR)
ISSN: 2454-1370

EFFECT OF GRAPE SEED OIL ON ALTERATIONS OF HEMATOLOGICAL PARAMETERS AND SEMEN FLUID QUALITY INDUCED BY DIAZINON IN MALE RATS

Muna H. AL-Saeed*,
Department of Physiology, Pharmacology and Chemistry, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

Abstract

Diazinon (DZN) is one of the organophosphorus (OP) insecticides widely used in local market, can affect animals even after exposure to a single dose. Grape seed oil (GSO), may be rich in more efficient antioxidants. Therefore, the aim of this study was conducted to evaluate the possible protective effect of grapes seed oil GSO in male rats exposed to diazinon at dose of DZN (50 mg/L) induced adverse effects on haematological and semen quality of male rats. The experimental 24 male albino rats were divided into four groups for 1 month. The rats of the first group were served as control. The experimental animals of the second group were exposed to diazinon (DZN). The animals of the third group were supplemented with grape seed oil and treated with DZN. The rats of the fourth group were supplemented with grape seed oil. Hematological such as Red blood corpuscles (RBC) count, hemoglobin (Hb) concentrations and hematocrit (Hct) showed significant reduction, while the value of white blood corpuscles (WBC) count was statistically increased in rats exposed to DZN. Hematological evaluations were chosen as indicators of DZN toxicity and protective role of grape seed oil. Moreover, the semen quality evaluations of the testis showed that DZN causes several severe alterations. Also, animals were dissected and the reproductive organs (epididymus and testes) were taken to measure fertility indices, oxidative parameters and testicular biomarkers. The results indicated DZN decreased testes and epididymus weights for this dose. This effect was dose - related and should be associated with decline in epididymus sperm count, percent of sperm motility, viability and maturity and increased abnormal sperm morphology.

Key words: Diazinon, Grape seed oil, RBC, WBC and Male rats.

1. Introduction

The pollution of environment by toxic chemicals is a global and chronic problem. Animal health risk due to exposure to chemical pollutants is constantly increasing. Insecticides form major toxic chemicals in environment. Scientifically, there is an obviously correlation between the exposure to insecticides and appearance of many diseases. Currently, the significance of natural products for health and medicine has been formidable (Hurtig *et al*., 2003). The organophosphate insecticide diazinon is commonly used in agriculture and households to control pest insects in soil, plants, fruit, and vegetable crops (Lari *et al*., 2013). Diazinon acts on the nervous system through the inhibition of the acetyl cholinesterase activity at the synapses and neuromuscular junctions and was manifested by overstimulation of acetylcholine receptors and impeded neurotransmission (Lotti, 2001). The ubiquitous distribution of both nicotinic and muscarinic cholinergic receptors together with induction of oxidative stress in various tissues

Corresponding author*: **Muna H. AL-Saeed
Received: 03.09.2016; *Revised*: 06.10.2016;
Accepted: 08.11.2016.

(Sarhan and Al-Sahhaf, 2011) may have genotoxic, immunotoxic, nephrotoxic, hepatotoxic and cardiotoxic effects (Salih, 2010). Diazinon suppresses reproductive function with endogenous hormonal disruption (Sallie *et al*., 1991; Sarabia *et al*., 2009), inducing histopathological alterations in testes and spermatogenic disturbances (Poet *et al*., 2004; Roegge *et al*., 2008; Razavi *et al*., 2013). Exposure to diazinon negatively affects sperm motility and DNA integrity, which may contribute to reduced semen quality and concomitant decreases in fertility (Banerjee *et al*., 1991; Sharma *et al*., 2005; Cakici and Akat, 2013).

Grape (*Vitis vinifera*) is one of the world's largest fruit crops and grape seed extract is a complex matrix containing approximately 40 % fiber, 16 % oil, 11 % proteins, and 7 % complex phenols including tannins in addition to sugars and mineral (Shi *et al*., 2003). Grape seed oil as an extract of the grape seed has many uses ranging from cooking (as a food additive), cosmetics and in controlling several diseases and wound healing potential (Shivananda Nayak *et al*., 2011). Nowadays, many scientific researchers have revealed that the grape seed oil has several health benefits and is considered as a good and potent antioxidant compound for its contents of polyphenols, flavonoids, unsaturated fatty acids and vitamin E. Grape seed oil (GSE) was reported to have many beneficial effects as antioxidant and free radical scavenger activity (El-Ashmawy *et al*.,2007) hepato-protective effect (Chan and Chang, 2006) or protection against ethanol-induced cell death (Dos Santos Freitas *et al*., 2008). Therefore, the present study was aimed to investigate the effect of grape seed oil on alterations of hematological parameters and semen fluid quality induced by diazinon toxicity in male rats.

2. Materials and Methods

Laboratory animals

A total of 24 healthy male albino rats of the Wistar strain (of about 180 - 220 g B.W.), and 48 female rate used only measurement fertility efficiency of male (mating) were obtained from the Animal House of College of Veterinary Medicine, Basrah University. The rats were left for one week period to enable acclimatization under standard hygienic conditions. Rats were fed on commercial pellet with free access to food and water. The experimental animals were housed 6 per cage in a room with 65 % humidity, 12:12 h light: dark cycle at ambient temperature of 20 ± 1 °C. Standard diet, commercial feed pellets and tap water were freely available. The principles of laboratory animal care were followed throughout the duration of experiment and instruction.

Preparation of Oil Extract of grape seed seeds

A (50 gm) of dried seeds powder was defatted with (500 ml) n-hexane for 16 hours by Soxhlet apparatus. The combined n-hexane extract was concentrated below 50 °C under reduced pressure in a rotary evaporator to get 7gm of yellow oil (Harborn, 1993).

Experimentation

The experimental animals were randomly distributed into five groups of six each. First group: Rats were untreated and served as normal control. Second group: Rats were orally administrated with 50 ml/L of DZN in normal saline, daily for 1 month. Third group: Rats were orally given grape seed oil at a dose of 1 ml/rat and after 4 hrs subjected to DZN at the same dose given to group 2, daily for 1 month. Fourth group: Rats were treated with grape seed oil at the same dose given to group 3, daily for 1 month.

Body weight and body weight gain measurement

The animals were weighed before and end period of the experiment.

Fertility test

Female rats were mated with adult male rats (2:1) in separated cages at 4:00 pm., vaginal smears were obtained in the next early morning for microscopic detection of sperms which indicates the first day of pregnancy. Positive females were isolated and date recorded.

Sampling

At the end of the experimental period, rats were fasted for 10 hrs, anesthetized using chloroform and blood samples were collected (control and treat animals) from the heart by tubes in plain tubes, and allowed to be clotted at room temperature and put in centrifuge at 5000 rm to obtain serum for hormonal assay and biochemical analysis such as MDA, GPx, SOD, ACP, ALP, AST and ALT and in heparinized tube for estimate hematological parameters.

Hormonal Assay

Serum samples were assayed for FSH, LH and testosterone using the Enzyme linked immunosorbent assay (ELISA) technique using the Fortress kit.

Semen Analysis

The seminal content of epididymis was obtained by cutting of cuda epididymis using surgical blades and squeezed into a clean petridish. This content was diluted 10 times with 2.9 % sodium citrate solution and thoroughly mixed to estimate percents of sperm progressive motility and sperm cell count as described by Mohammad Reza *et al*. (2005). One drop of sperm suspension was withdrawn, smeared on clean glass slide and stained by Eosin-Nigrosin stain. The stained seminal smears were examined microscopically to determine percents of sperm viability (alive/dead ratio) and morphology as described by Laing (1979).

Progressive sperm motility

This was done immediately after the semen collection. Semen was squeezed from the caudal epididymis onto a pre-warmed microscope slide (37 ˚C) and two drops of warm 2.9 % sodium citrate was added, the slide was then covered with a warm cover slip and examined under the microscope using 400 X magnification. Ten fields of the microscope were randomly selected and the sperm motility of 10 sperms was assessed on each field. Therefore, the motility of 100 sperms was assessed randomly. Sperms were labelled as motile, sluggish or immotile. The percentage of motile sperms was defined as the number of motile sperms divided by the total number of counted sperms (i.e. 100) (Laing, 1979).

Sperm viability (Live/dead ratio)

This was done by adding two drops of warm Eosin/Nigrosin stain to the semen on a pre-warmed slide, a uniform smear was then made and dried with air. The stained slide was immediately examined under the microscope using 400X magnification. The live sperm cells were unstained while the dead sperm cells absorbed the stain. The stained and unstained sperm were counted and the percentage was calculated (Morel *et al*., 1998).

$$\textbf{Live sperm \%} = \frac{\textbf{Live sperm}}{\textbf{Total sperm count}} \text{ x } 100$$

$$\textbf{Dead sperm \%} = \frac{\textbf{Dead sperm}}{\textbf{Total sperm count}} \text{ x } 100$$

Sperm maturation by aniline - blue

Nuclear maturation was evaluated by aniline-blue stain, according to Morel *et al.* (1998). Sperm nuclei that stained with blue color were considered to be immature. But, nuclear mature sperm was not stained with aniline - blue. The percentage of immature sperm was calculated from the observation of one hundred sperm preparation from each group.

Sperm Morphology

A drop of Nigrosin - Eosin stain was added to the sperm suspension and kept for 5 min at 37 ˚C. After that a drop of sperm suspension was placed

on a clean slide and spread gently to make a thin film. The film was air dried and then observed under a microscope for changes in sperm morphology, according to the method of Feustan *et al.* (1989). The criteria chosen for head abnormality were amorphous, pin and short head. For tail, the abnormalities recorded were; coiled flagellum, bent flagellum, bent flagellum tip. The result are the percentage overall abnormal form.

Sperm count

This was done by removing the caudal epididymis from the right testes and blotted with filter paper. The caudal epididymis was immersed in 5 ml formol-saline in a graduated test tube and the volume of fluid displaced was taken as the volume of the epididymis. The caudal epididymis and the 5 ml formol-salline were then poured into a mortar and homogenized into a suspension from which the sperm count was carried out using the improved Neubauer haemocytometer under the microscope (Morel *et al*., 1998).

Histology examination

After removing the liver and kidney they were immediately fixed in Bouin's fluid for 12 hours and the Bouin's fixative was washed from the samples with 70 % alcohol. The tissues were then cut in slabs of about 0.5 cm transversely and the tissues were dehydrated by passing through different grades of alcohol: 70 % alcohol for 2 hours, 95 % alcohol for 2 hours, 100 % alcohol for 2 hours, 100 % alcohol for 2 hours and finally 100 % alcohol for 2 hours. The tissues were then cleared to remove the alcohol; the clearing was done for 6 hours using xylene. The tissues were then in filtrated in molten Paraffin wax for 2 hours in an oven at 57 $^{\circ}$C, thereafter the tissues were embedded. Serial sections were cut using rotary microtone at 5 microns (5 μm). The satisfactory ribbons were picked up from a water bath (50 $^{\circ}$C – 55 $^{\circ}$C) with microscope slides that had been coated on one side with egg albumin as an adhesive and the slides were dried in an oven. Each section was deparaffinized in xylene for 1 minute before immersed in absolute alcohol for 1 minute and later in descending grades of alcohol for about 30 seconds each to hydrate it. The slides were then rinsed in water and immersed in alcoholic solution of hematoxylin for about 18 minutes. The slides were rinsed in water, then differentiated in 1 % acid alcohol and then put inside a running tap water to blue and then counterstained in alcoholic eosin for 30 seconds and rinsed in water for a few seconds, before being immersed in 70 %, 90 % and twice in absolute alcohol for 30 seconds each to dehydrate the preparations. The preparations were cleared of alcohol by dipping them in xylene for 1 minute. Each slide was then cleaned, blotted and mounted with DPX and cover slip, and examined under the microscope. Photomicrographs were taken at 40X, 100X and 400X magnifications (Luna, 1993).

Statistical Analysis:

The data were analyzed by SPSS software using one way variance analysis ANOVA, Version 16. In all tests, a P - value of <0.05 was considered statistically significant (SPSS, 2001).

3. Result

Effect of Grape seeds oil on Body weight and body weight gain in male Rats Treated with Diazonoin

The obtained results in Table - 1 revealed significant decrease ($P \leq 0.05$) in body weight and body weight gain of male rats treated with DZN alone group at dose 50 ml/L compared with control group, DZN + GSO group and GSO alone at a dose 1ml/rat while the results were showed non-significant ($p<0.05$) decrease body weight and body weight gain male rats treated with treated with DZN + GSO group compared with control group but that showed significant ($p<0.05$) increase body weight and body weight gain of male rats treated with treated with GSO group compared with control group and another groups.

Effect of Grape seeds oil on fertility efficiency in male rats mating treated with Diazonoin with female untreated

The results in Table - 2 observed that the effect of grape seeds oil on fertility efficiency in male rats treated with Diazonoin mating with female untreated. The results were showed increase pregnancy rate in female mating with male rats treated with grape seeds oil compared with control and significant ($p<0.05$) increase in Number of litter size and improvement of fertility efficiency in male rats treated with DZN + GSO mating with female untreated compared with group treated DZN alone.

Effect of Grape seeds oil on RBC counts and RBC Index in male Rats Treated with Diazonoin

The obtained results in Table - 3 revealed significant decrease ($P\leq0.05$) in RBC, Hb and PCV % of male rats treated with DZN alone group at dose 50 ml/L compared with control group, DZN + GSO group and GSO alone at a dose 1 ml/rat while the result showed non-significant change in RBC, Hb and PCV of male rats treated with DZN + GSO group and GSO alone compared with control group. The results of MCV revealed significant increase ($P\leq0.05$) in MCV of male rats treated with DZN alone at dose 50 ml/L compared with control group, DZN + GSO group and GSO alone at a dose 1 ml/rat while the result showed non-significant change in MCV and at a dose 1 ml/rat compared with control group and non-significant change of MCV in male rats treated with DZN + GSO group and GSO alone at a dose1ml/rat compared with control group. The results of MCH revealed non-significant changes between treated groups compared with control group. The results of MCHC revealed significant decrease ($P\leq0.05$) in MCHC of male rats treated with DZN alone at dose 50 mg/kg B.W compared with control group, DZN + GSO group and GSO alone at a dose 1 ml/rat while the result showed non-significant change of MCHC in male rats treated with DZN + GSO group and GSO alone at a dose1ml/rat compared with control group.

Effect of Grape seeds oil on Total WBC counts and Differential in male Rats Treated with Diazonoin

The obtained results in Table - 3 revealed the significant decrease ($P\leq0.05$) in WBC of male rats treated with DZN alone at doses 50 ml/L compared with control group and another treated groups while the results showed significant increase in WBC of male rats treated with GSO alone at dose 1 ml/rat compared with control group and another treated groups. The results of lymphocyte % and monocyte % showed significant decrease ($P\leq0.05$) in male rats treated with DZN alone at dose 50 ml/L compared with control group and another treated groups and this results of lymphocyte % showed non-significant change in male rats treated with GSO at dose 1 ml/rat compared with control group. The results of Neutrophils % showed significant increase ($P\leq0.05$) in male rats of all treated group compared with control group while the result of Neutrophils % showed non-significant ($P\leq0.05$) in male rats all treated groups compared between them groups. The results of Eosinophil % and Basophile % showed significant increase ($P\leq0.05$) in male rats treated with DZN group compared with control group and significant decrease ($P\leq0.05$) in male rats treated GSO alone group and (DZN + GSO) group compared with control group and (DZN).

Effect of Grape seeds oil on physical properties of semen analysis in male rat treated with diazonoin

The results in Table - 4 observed that the effect of Grape seeds oil on physical properties of semen analysis in male rabbits. The results were showed significant ($p<0.05$) increase in semen volume, sperm motility, sperm concentration, total sperm cell/ejaculate, live – dead sperm and showed significant decrease sperm abnormalities compared with control group.

Table - 1: Effect of Grape seeds oil on weight and body weight gain in male rats treated with Diazonoin. Mean ± SD; N=6

Parameters / Treatment	Initial Body weight (G)	Final Body weight (G)	Body weight gain (G)
Control (Normal Saline) 0.9 % NaCl	192±12.03 A	205±20.41 AB	13±4.11 B
DZN (50 ml/L)	188 ±17.06 A	135±9.32 C	53±7.36 C
DZN + GSO	187±13.34 A	195±14.5 B	8±0.21 B
GSO 1 ml/rat	189±16.41 A	220±21.13 A	31±9.01 A

N = Number of animals; A, B, C = Differences between groups, P≤0.05 vs control.

Table - 2: Effect of Grape seeds oil on fertility efficiency in male rats mating treated with Diazonoin with female untreated. Mean ± SD; N = 6

Parameters / Treatment	No. of female pregnancy	Pregnancy rate (%)	No. Litter size	Embryo mortality
Control (Normal Saline) 0.9 % NaCl	5	83.33 %	9±1.46 AB	-
DZN (50 ml/L)	2	33.33 %	3±1.13 C	Abortion
DZN + GSO	4	66.66 %	7±1.8 B	-
GSO 1 ml/rat	6	100 %	12±2 A	-

N = Number of animals; A, B, C = Differences between groups, P≤0.05 vs control.

Table - 3: Effect of Grape seeds oil on RBC counts and RBC Index in male Rats Treated with Diazonoin (Mean ± SD) (n=6)

Parameters / Treatment	RBC × 10^6/µL	Hb g/dl	PCV (%)	MCV Fl	MCH pg	MCHC %
Control (Normal saline)	7.41±0.24 A	13.03±0.32 A	38.95±0.33 A	48.50±2.25 B	17.00±0.50 NS	34.16±2.85 AB
DZN (50 ml/L)	4.33±0.60 B	8.33±1.632 B	30.83±2.48 B	52.83±4.16 A	16.08±1.42 NS	28.45±1.02 C
DZN + GSO	7.14±0.37 A	12.88±0.53 A	38.90±0.37 A	48.83±1.16 B	16.88±0.65 NS	32.83±2.04 B
GSO 1 ml/rat	7.46±0.43 A	13.41±0.34 A	43.61±2.49 A	47.90±5.93 B	17.01±0.17 NS	35.83±1.47 A

N = Number of animals; A, B, C = Differences between groups, P≤0.05 vs control

Table – 3: Effect of Grape seeds oil on Total WBC counts and Differential in male Rats Treated with Diazonoin (Mean ± SD) (n = 6)

Parameters / Treatment	WBC × 10^3/µL	Lym%	Mono%	Neutro%	Eso%	Baso%
Control (Normal saline)	5.58±0.73 B	60.08±4.29 A	4.20±0.37 A	35.76 ±4.49 B	0.76±0.06 B	0.28±0.04 B
DZN (50 ml/L)	3.06±0.37 C	43.60±3.41 B	2.30±0.37 B	48.50±3.17 A	1.81±0.03 A	2.69±0.08 A
DZN + GSO	5.70±0.37 B	52.66±4.17 A	4.38±0.37 A	44.00±5.54 A	0.00±00 C	00.00±0.00 C
GSO 1 ml/rat	6.61±0.30 A	54.83±4.02 A	4.90±0.25 A	42.20±3.50 A	0.00±0.00 C	0.00±0.00C

N=number of animals, A,B,C= differences between groups, P≤0.05 vs. control.

Table - 4: Effect of Grape seeds oil on physical properties of semen analysis in male rat treated with Diazonoin, Mean ± SD; N = 6

Parameters / Groups	Control (Normal Saline) 0.9 % NaCl	DZN (50 ml/L)	DZN+GSO	GSO 1ml/rat
Semen volume(ml)	0.55±0.0072 B	0.35±0.0013 C	0.50±0.004 B	0.88±0.015 A
Semen colour	Creamy	Yellowish	Creamy	Creamy
Mass activities	++	----	++	+++++
Sperm motility %	78.15±2.31 B	50.89 ±9.36 C	77.35±8.44 B	95.14±7.41A
Sperm concentration ($\times10^6$/ml)	6.12±0.24 B	4.20±0.14 C	5.94±0.13B	8.67±1.09 A
Total sperm cell/ejaculate ($\times10^6$/ml)	3.74±0.36 B	2.54±0.03 C	3.24±0.08 B	5.98±0.69 A
Live-dead sperm ratio	70:30±4.89B	40:60±7.49 C	65:35±5.63B	95: 5±9.82 A
Sperm abnormalities	15.69±3.7 B	35.98±11.79A	16.42±3.48	4.72±0.22 C

N = Number of animal; A, B, C = Differences between groups, P≤0.05 vs control

Table - 5: Effect of Grape seeds oil on biochemical parameter in male rat treated with diazonoin; Mean ± SD, N = 6

Parameters / Treatment	MDA (nmol/ml)	GPx (mmol/L)	SOD U/dL	ALP U/L	ACP U/L	ALT U/L	AST U/L
Control (Normal Saline) 0.9 % NaCl	20.37±0.45 C	19.13±0.43 A	87.6 ±5.75 A	30.4±6.2 B	29.3±7.11 B	47.2±1.05 C	29.2±3.4 C
DZN (50 ml/L)	32.09±0.5 A	4.34± 0.03 C	56. 36±0.13 B	60.41± 11.34 A	74.20±2.8 9A	79.2±3.15 A	64.1±1.2 A
DZN + GSO	25.08±0.13 B	12.32±0.59B	89.45±0.28 A	35.22±8.7 4 B	31.47±3.8 6 B	56.3 ± 6.29 B	35.7±6.7 B
GSO (1 ml/rat)	19. 98± 0.8 C	20.17±3.05A	90.29±0.15 A	26.40±5.9 B	27.4±3.18 B	42.92±3.1 5 C	26.5±7.8 C

N = Number of animal; A, B, C = Differences between groups, P≤0.05 vs control.

Table – 6: Effect of Grape seeds oil on FSH, LH and Testosterone in male rat treated with diazonoin; Mean ± SD, N=6

Parameters / Treatment	FSH (mlU/ml)	LH (mlU/ml)	Testosterone (ng/ml)
Control (Normal Saline) 0.9 % NaCl	2.80±0.013 A	5.24±0.007 A	1.48±0.013 A
DZN (50 ml/L)	1.09±0.025 C	1.6±0.018 C	0.24±0.014 C
DZN + GSO	2.49±0.014 B	4.75±0.021 B	1.16±0.015 B
GSO (1 ml/rat)	2.85±0.012 A	5.40±0.01 A	1.66±0.020 A

N = Number of animal; A, B, C = differences between groups, P≤0.05 vs control

Effect of Grape seeds oil on biochemical parameter in male rat treated with diazonoin

The results shown in Table - 5 revealed that, levels of serum MDA, ALP, ACP, ALT and AST in male rats treated with diazonoin significant ($p<0.05$) increase compared with control group, grape seed oil and DZN + GOS while activities of GPx and SOD in male rats treated with DZN showed significant ($p<0.05$) decrease compared with control and another treated group.

Effect of Grape seeds oil on FSH, LH and Testosterone in male rat treated with diazonoin

The results in Table - 6 observed that that the effect of Grape seeds oil on FSH, LH and testosterone in serum male rats. The results were showed significant ($P<0.05$) decrease of FSH, LH and testosterone level in serum male rats treated with DZN alone at doses 50 ml/L compared with control group and other groups while the results were showed non-significant ($P >0.05$) changes of testosterone level in serum male rats treated with oil Grape seeds oil compared with control.

Sperm examination

Sperms of rats (control). Showing almost of sperms normal, live and decrease number of mature sperm while the sperm of rats treated with DZN alone showing large number of sperms dead and large number of different types of abnormalities sperm coiled tail, double tail, Only Head when stained with eosin and negrosin but sperms of rats treated with (DZN + GSO). Showing ameliorating in morphology of sperm normal, increase number of live sperm and mature sperm and decreased in number of dead sperm when stained with eosin and negrosin stain also the sperm of rat treated with GSO showing all sperm normal, increase number of live sperm and mature sperm and decreased in number of dead sperm when stained with eosin and negrosin stain.

Histological Examination

Liver

Histological section of liver of control male rats. Show normal hepatocyte normal portal vein, sinusoid while liver of rat treated with DZN alone. Showing irregular arrangement of hepatocyte, enlarged spaces of sinusoid, minimal diffuse vacuolation of hepatocytes but section of liver of male rats treated with DZN + GSO. Show enlargement of hepatic cells, pyknotic cells as well as some dilated of hepatic sinusoid also histological section of liver of male rats treated with GSO alone. Show normal hepatocyte normal central hepatic vein, siunsiod.

Kidneys

Histological section of kidneys of control male rats. Show normal glomeruli, normal renal cortical tubules and normal epithelial cells lining of the renal tubules. While kidneys of male rat treated with DZN alone. Show the changes included infiltrations of inflammatory cells,

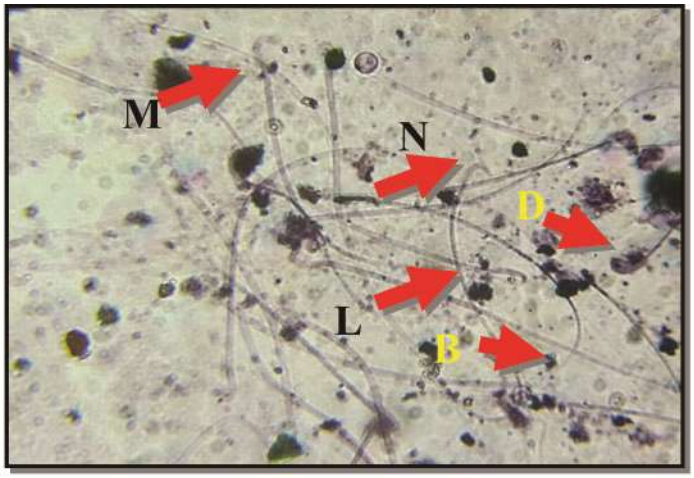

Fig (1):-Sperms of rats (control). Showing almost of sperm normal(N), live (L) and mature(M) and present some of sperms dead (D)and abnormalities (B)tail coil. Stained with E &N, 400X.

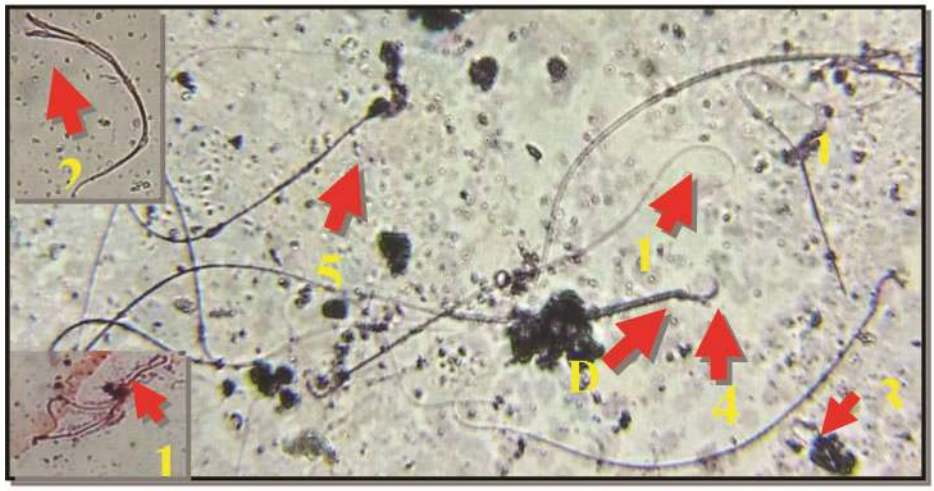

Fig (2):-Sperms of rats treated with (DZN). Showing Oligosperm and almost of sperm dead (D), large number of different types of abnormalities sperm 1-Coiled tail 2-Double–head 3-OnlyHead 4-Big-head 5- only Tail. Stained with E&N,400X.

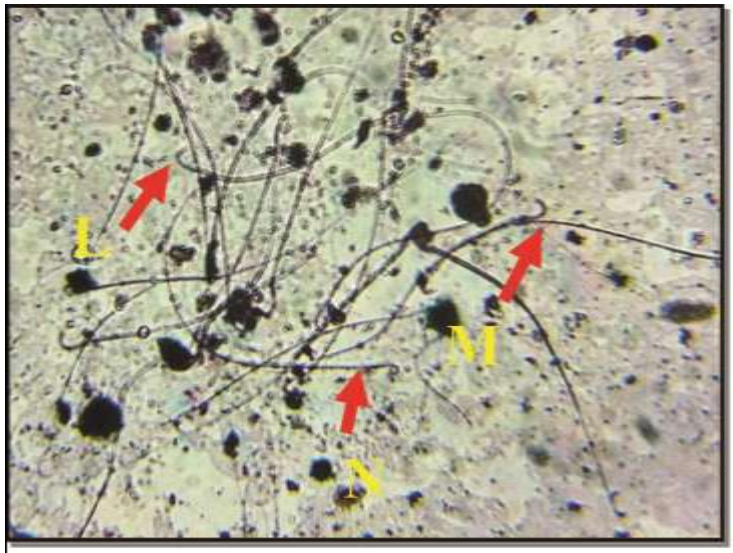

Fig (3):-Sperms of rats treated with (DZN+GSO). Showing almost of sperm normal(N), live (L) and mature(M) and present some of sperms dead and abnormalities. Stained with E &N, 400X

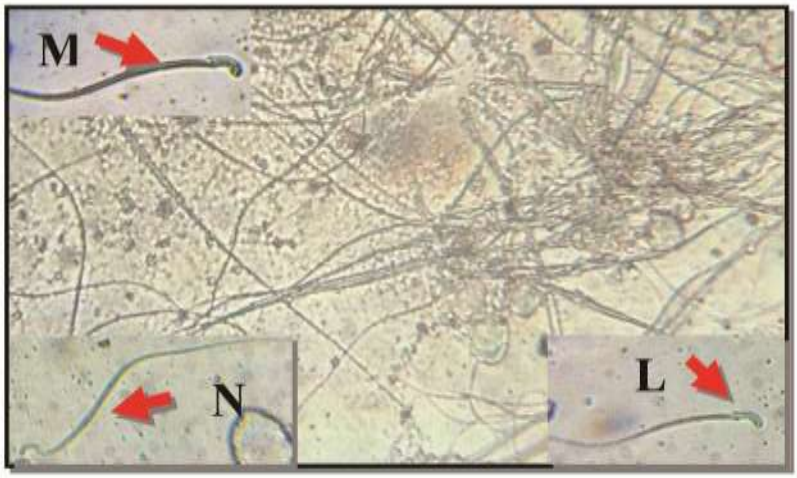

Fig (4):-Sperms of rats treated with (GSO). Showing almost of sperm normal(N), live (L) and mature(M). Stained with E &N, 400X.

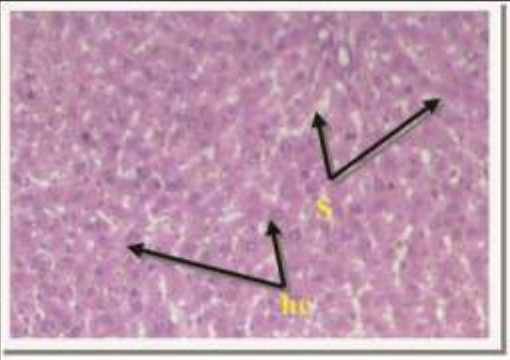

Fig.5: Histological section of liver of control rats. Showing normal hepatocyte (hc) normal portal vein, normal central vein, siunsiod (S), stained with (H&E)100X.

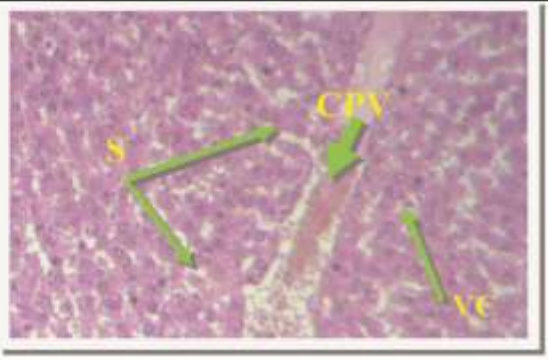

Fig.6: Histological Section of liver of rats treated with DZN. Showing congestion of portal vein (CPV), irregular arrangement of hepatocyte, pyknotic cells as well as some enlargement of hepatic sinusoid(S) and vacuolated cytoplasm of hepatocyte(VC), stained with (H&E)100X.

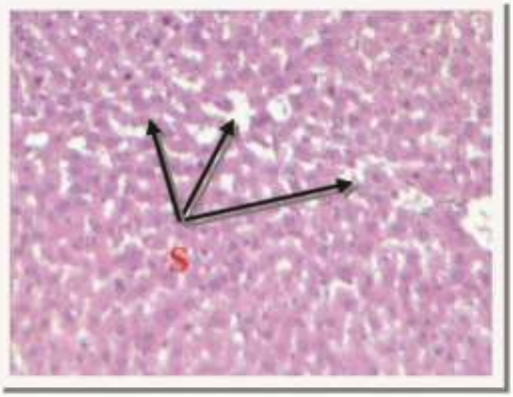

Fig.7: Histological Section of liver of rats treated with DZN+GSO. Showing enlargement of hepatic cells, pyknotic cells as well as some dilated of hepatic sinusoid(S), stain (H&E)100X.

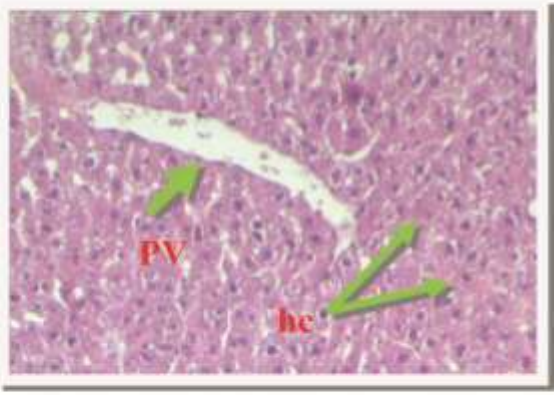

Fig.8: : Histological Section of liver of rats treated with GSO. Showing normal hepatocyte (hc) normal portal vein (PV), siunsiod (S), stain(H&E)100X.

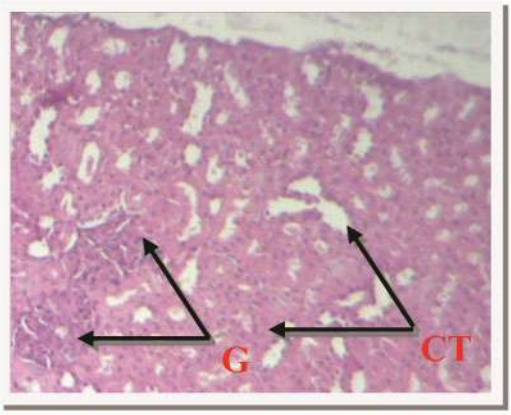

Fig.9:-Histological section kidneys of control male rats. Showing normal glomeruli (G) and normal renal cortical tubules (CT), stained with (H&E) 100X.

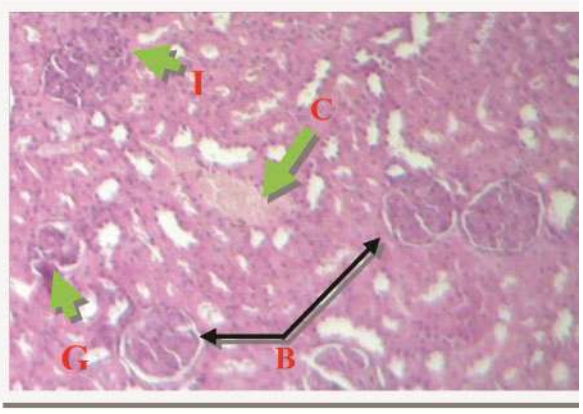

Fig.10:-Histological section kidneys of mal rats treated with DZN alone. Showin vascular congestion and narrowed Bowman' space between Bowman's Capsule (B)an glomeruli , some atrophy of glomeruli (C and infiltrations of inflammatory cells (I stained with (H&E) 100X.

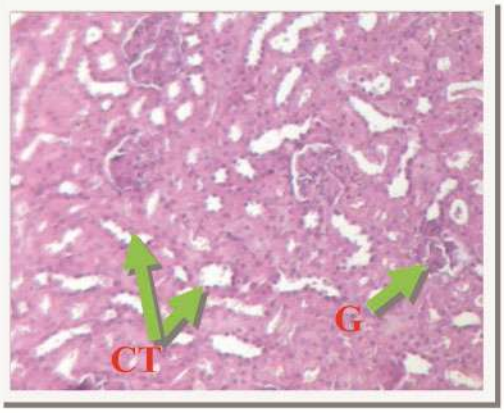

Fig.11:-Histological section kidneys of male rats treated with DZN+GSO. Showing almost of glomeruli normal, normal renal cortical tubules (CT) and some atrophy of glomeruli (G) stained with (H&E) 100X.

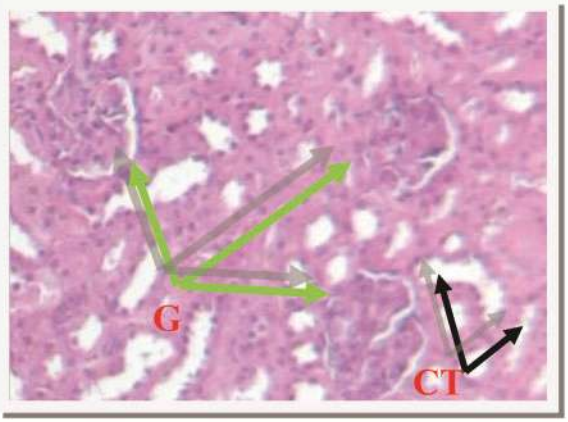

Fig.12:-Histological section kidneys of male rats treated with GSO alone. Showing normal glomeruli and normal renal cortical tubules (CT), stained with (H&E) 400X.

vascular congestion and narrowed Bowman's space, glomeruli but histological section of kidneys of male rats treated with DZN + GSO. Show almost of glomeruli normal, normal renal cortical tubules and some atrophy of glomeruli also histological section of kidneys of male rats treated with GSO alone.

4. Discussion

The present study was aimed to investigate the effect of grape seed oil on alterations of hematological parameters and semen fluid quality induced by diazinon toxicity in male rats. Grape seed oil caused improvement of heamatological parameters due to have many beneficial effects as antioxidant and free radical scavenger activity. The grape seed oil has several health benefits and

is considered as a good and potent antioxidant compound for its contents of polyphenols, flavonoids, unsaturated fatty acids and vitamin E (Shivananda Nayak *et al*., 2011).

The grape seed oil caused amelioration of physical and chemical properties of semen rats. It caused significant increase in sperm viability as well as significant increase in the percentage of morphologicaly normal sperm cells in the treated rats. This could be due to the ability of grape seed oil to either interfere with the spermatogenic processes in the seminiferous tubules, epididymal functions or activities of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins which may result in activation of spermatogenesis (William, 2001; Bowman and Rand,1985).

Grape seed oil caused significant increase in sperm count of the treated rats which could be as a result of increase in plasma levels of testosterone since this hormone has been reported to be important in the initiation and maintenance of spermatogenesis (Christensen, 1975). These parameters in male rats are regulated by LH and FSH. The spermatogenesis stimulates by FSH which binds with receptors in sertoli cells while the LH stimulates the production of testosterone in leyding cells, which may act on sertoli and pertitubular cells of testosterone (O′Donnel *et al*., 1994).

Rats treated with grape seed oil presented with improvement of seminiferous tubules and spermatogenesis. Testosterone stimulates growth and secretary activity to the reproductive organs (Singh *et al*., 1995), so these hormones in this study could increase the number and function of germinal and somatic cells of testis. This suggests that grape seed oil had ameliorating effect on the exocrine function of the testes.

Reference

1) Banerjee, B. D., Seth, V., Bhattacharya, A., Pahsa, S.T and Chacraborty, A. K. 1999. Biochemical effects of some pesticides on lipid peroxidation and free radical scavengers. *Toxicology Letters*, 107: 33 – 47.
2) Bowman, W. C. and Rand, M. J. 1985. The reproductive system and drugs affecting the reproductive systems. Textbook of pharmacology, 2nd edition, 20: 1 - 8.
3) Cakici, O and Akat, E. 2013. Effects of oral exposure to diazinon on mice liver and kidney tissues: biometric analyses of histopathologic changes. *Annals of Quantitative Cytology and Histology*, 35: 7 – 16.
4) Chan, W. H and Chang, Y. J. 2006. Dosage effects of resveratrol on ethanol-induced cell death in the human K562 cell line. *Toxicology Letters*, 161: 1 - 9.
5) Christensen, A. C. 1975. Leydig cell: In: Handbook of Physiology, edited by P.O. Greep and E.B. Astwoods. Washington D C American Physiological Society.
6) Dos Santos Freitas, L., Jacques, R. A., Richter, M. F., Silva, A. L and Caramao, E. B. 2008. Pressurized liquid extraction of vitamin E from Brazilian grape seed oil. *Journal of Chromatography*,1200: 80 - 83.
7) El-Ashmawy, I. M., Amal, S and Osama, M. S. 2007. Effects of marjoram volatile oil and grape seed extract on ethanol toxicity in male rats. *Basic Clinical Pharmacology and Toxicology*,101: 320 – 327.
8) Feustan, M. H., Bodnai, K. R. and Kerstetter, S. L. 1989. Reproductive toxicity of 2-methoxy ethanol applied dermally to occluded and non-occluded sides in male rats. *Toxicology and Applied Pharmacology*, 100: 145 – 165.
9) Harborn, J. B. 1993. Phytochemical methods. Guide to modern technique in Plant Analysis. 4thEd., New York, USA. Chapman and Hall.
10) Hurtig, A. K., San Sebastian, M., Soto, A., Shingre, A., Zambrano, D and Guerrero, W. 2003. Pesticide use among farmers in the Amazon basin of Ecuador. *Archives of Environmental Health*, 58: 223 – 228.
11) Laing, J. A. 1979. Fertility and infertility in domestic animals. 3rd edition Bailliere Tindall, a division of Cassell Lt.

12) Lari, P., Abnous, K., Imenshahidi, M., Rashedinia, M., Razavi, M and Hosseinzadeh, H. 2013. Evaluation of diazinon-induced hepatotoxicity and protective effects of crocin. *Toxicology and Indian Health* (in press).
13) Lotti, M. 2001. Clinical toxicology of anticholinesterase agents in humans. Handbook of Pesticide Toxicology, 2nd Ed., Academic Press, USA, 2001. p. 1043.
14) Luna, L. G. 1993. Manual of histology staining methods of the Armed Forces Institute of Pathology. 3rd ed., New York, McGraw Hill.
15) Mohammad Reza, P., Farzaneh, D., Taherch, T. K and Zoherb, P. P. 2005. The effects of hydroalcholic extract of *Actinidia chinensis* on sperm count and motility, and blood levels of estradiol and testosterone in male rats. *Achieves of Iranica Medicin*e, 8 (3): 211 - 216.
16) Morel, B. S., Mercier, S and Roux, C. 1998. Inter individual variations in the disomy frequencies of human spermatozoa and their correlation with nuclear maturity as evaluated by aniline blue staining. *Fertilization and Sterilization*, 69: 1122 - 1127.
17) O′Donnel, L., Mc Lanchlan, R. L., Wreford, N. G and Robertson, D. M. 1994. Testosterone promotes the conversion of round spermatids between stages vii and viii of the rat spermatogenic cycle. *Endocrinology*, 135: 2608 - 2614.
18) Poet, T. S., Kousba, A. A., Dennison, S. L and Timchalk, C. 2004. Physiologically base pharmacokinetic or pharmacodynamic model for the organophosphorus pesticide diazinon. *Neurotoxicology*, 25: 1013 – 1030.
19) Razavi, B. M., Hosseinzadeh, H., Movassaghi, A. R., Imenshahidi, M. and Abnous, K. 2013. Protective effect of crocin on diazinon induced cardiotoxicity in rats in subchronic exposure. *Chemical and Biological Interaction*, 203: 547 – 555.
20) Roegge, C. S., Timofeeva, O. A., Seidler, F. J., Slotkin, T. A and Levin, E. D. 2008. Developmental diazinon neurotoxicity in rats: later effects on emotional response. *Brain Research Bulletin,*75: 166 – 172.
21) Salih, E. M. A. 2010. Toxic effect of dimethoate and diazinon on the biochemical and hematological parameters in male rabbits. *Jordan Journal of Biological Sciences,* 3: 77 – 82.
22) Sallie, R., Tredger, J. M and William, R. 1991. Drug and liver. *Biopharmacy and Drug Disposal,*12: 251–259.
23) Sarabia, L., Maurer, I and Bustos-Obregon, E. 2009. Melatonin prevents damage elicited by the organophosphorous pesticide diazinon on the mouse testis. *Ecotoxicology and Environmental Safety*,72: 938 – 942.
24) Sarhan, O. M. M and Al-Sahhaf, Z. Y. 2011. Histological and biochemical effects of diazinon on liver and kidney of rabbits. *Life Science Journal*, 8: 1183 – 1189.
25) Sharma, Y., Bashir, S., Irshad, M., Gupta, S. D and Dogra, T. D. 2005. Effects of acute dimethoate administration on antioxidant status of liver and brain of experimental rats. *Toxicology*, 206: 49 – 57.
26) Shi, J., Jianmel, Y., Joseph, E. P and Yukio, K. 2003. Polyphenolics in grape seeds biochemistry and functionality. *Journal of Medicinal Food*, 6: 291– 299.
27) Shivananda Nayak, B., Dan Ramdath, D., Marshall, J. R., Isitor, G., Xue, S and Shi, J. 2011. Wound healing properties of the oils of *Vitis vinifera* and *Vaccinium macrocarpon*. *Phytotheraphy Research*, 25: 1201 – 1208.
28) Singh, J., O′Neill, C and Handelsman, D. J. 1995. Induction of spermatogenesis by androgens in gonadotropin - deficient mice. *Endocrinology*, 136: 5311 – 5321.
29) SPSS Statistical Packages for the Social Sciences. 2001. Statistical soft ware for windows version 13.0 Microsoft. SPSS, Chicago, IL,USA.
30) William, K. W. 2000. Hormones and Hormone antagonists. In: Remington, The Science and Practice of Pharmacy, Volume - 11, 20th edition 77: 1390-1391.