Global Veterinaria 7 (4): 370-380, 2011
ISSN 1992-6197

Protective Effect of *Selenium* on *Diazinon* Induced Determination Impact on the Testes in Mature Male Rats

[1]*Karimi Ali,* [2]*Najafi Golam Reza,* [3]*Salami Syamak,* [4]*Hoshyari Aref,* [5]*Hosseini Ehsan,*
[6]*Rezazadeh Leila,* [6]*Babaei Mohammad,* [6]*Najafi Ali,* [7]*Kardan Davoud and* [8]*Ghasemzadeh Esmaiel*

[1]Department of Comparative Histology and Embryology, Urmia University, Urmia, Iran
[2]Department of Anatomy, Faculty of Veterinary Medicine, P.O. Box: 1177, Urmia University, Urmia, Iran
[3]Department of Basic Science, Faculty of Medical Science, Urmia Medical University, Urmia, Iran
[4]Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[5]Department of Physiology, Faculty of Veterinary Medicin, Urmia University, Urmia, Iran
[6]Faculty of Veterinary Medicine, Urmia University, Urmia, Iran
[7]Faculty of Veterinary Medicine, Urmia Branch, Islamic Azad University, Urmia, Iran
[8]Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz, Iran

Abstract: To evaluate the effect of *selenium* (SE) as a potential antioxidant on *diazinon* (DZN) induced histopathological damages, 54 mature male rats were used. The animals were assigned into 3 groups including control-sham, DZN alone and SE+DZN groups. The control-sham group received corn oil and the animals in DZN and SE+DZN groups received diazinon 300 mg /kg and selenium 6 µg/rat [8], orally, once a day for 60 days respectively. Histological and histochemistry studies were conducted to uncover any cellular damages which DZN alone and the treatment regimens might exert on testicular tissue and sperm contents. Light microscopic analyses manifested that in DZN alone administrated cases sever germinal cells degeneration and depletion were occurred. Moreover in DZN alone received rat's spermatocytigenesis cell series cytoplasmic carbohydrate ratio was decreased and cytoplasmic lipid accumulation was elevated. Immature, immotile, death and DNA damaged sperms number increased in DZN animals. However in SE+DZN all above abnormalities were decreased.

Key words: Diazinon · Selenium · Testis · Sperm

INTRODUCTION

Diazinon (DZN) is an insecticide witch is widely used in agriculture and to control pests in the environment, this compound can be highly toxic for animals and human kind [1, 2]. DZN is characterized in organophosphate (OP) agrochemicals [3]. These compounds exert inhibiting effect on cholinesterase (ChE) activity by phosphorylation the catalytic site of the enzymes [4]. The DZN intoxication may be occurred either by direct inhalation exposure or by dermal direct contact, as most of the OPs, which are capable to be absorbed through the skin [5]. OP compounds are proofed to have toxic effects on reproductive tract in male rats and histologically they induce severe focal necrosis and/or degeneration of the germ cells in the seminiferous tubules (STs) associated with remarkable tubular atrophy [6-8]. Therefore, the health hazards on human of this class of chemicals have attracted the attention of many investigators. Thus the first purpose of the present study was to evaluate the histological adverse effect of DZN on male genital system and to investigate morphologically quantitative and qualitative alterations in sperms of male rats which were experimentally induced with DZN.

Selenium (SE) has received considerable attention as an essential micronutrient for both animal and human beings. It has been detected that this compound functions in the active site of glutathione peroxidase (GSH-Px) [9]. Nowadays it has been reported that the SE is a potentially antioxidant agent [10, 11]. Previous studies reported that the synthetic antioxidant agents (vitamin E, melatonin) and/or natural potentially antioxidants

Corresponding Author: Ghasemzadeh Esmaiel, Faculty of Veterinary Medicine, Tabriz Branch, Islamic Azad University, Tabriz Iran. Tel: +989358915501.

(Glycyrrhiza glabra, liquoric plant, zataria moltiflora) have beneficial effects on different toxic compounds toxicities [12-14] On the other hand according to several studies, the OP pesticides and/or insecticides exposed animals and human, were manifested with considerable oxidative stress on their genital tract [15-17]. Thus the second purpose of the current study was to analyze the protective effect of selenium on DZN induced oxidative stress in rats.

MATERIALS AND METHODS

Animals: 54 mature male Wister rats, 12 weeks old weighting 20 ± 200 gr were used. The rats were purchased from the Animal Resources and they were acclimatized in an environmentally controlled room (temperature, 20-23°C and 12h light/12h dark). Special plates with tap water were given to all groups.

Experimental Design: Following a week acclimation the animals were assigned into three groups as control-sham (n=6) and tests; DZN alone and DZN+SE administrated groups (n=24 for each group). All animals from mentioned groups prior to launching of experiment and after the latest step of the treatment were weighted to evaluate any changes in body weight gain (BWG). Animals in control-sham group received the corn oil (0.2 ml/day) and the rats in the test groups were administrated DZN at dose of 300 mg /kg, b.w., orally, once a day for 60 days. The all groups were sampled on days 24, 52 and 60 after dosing.

Testicular Weight Determination: All rats on days 24,52 and 60 following anesthesia with Ketamine5% (Iran), 40 mg/kg, i.p. and xylazine 2% (Germany) 5 mg/kg, i.p. were euthanized by using CO_2 gas in a special device and immediately following weighting of total body weight (BW) the testicles were excised free of surrounding tissues and weighed on a C.F600 scale (Delta Range, Tokyo).

Histopathological and Histochemical Examination: The all testes were fixed in 10% formalin fixative for histological investigations and subsequently embedded in paraffin. Sections (5-7 µm) were stained with Iron-Weigert for detection of germinal cells nucleuses in the testis in order to histopathological assessment. All of the specimens were studied by multiple magnifications (400X and 1000X). For the quantification of cells and their dimensions we used 100µm morphometrical lens-device (Olympus, Germany). The dimensions were expressed in µm.

One half of the right and left testes were used freshly in order to histochemical study. For this purpose frozen sections (15µm) were prepared from samples. Sections were stained with techniques such as periodic acid shift (PAS) in order to detect carbohydrate changes and Sudan black B (SB) to be able to detect the range of lipid accumulation in damaged germinal cells.

Epididymal Sperm Content, Quantitative Sperm Mortality and Morphology: Epididymides were separated carefully from the testicle under a 10 time magnification provided by Stereo Zoom Microscope (model TL_2, Olympus Co., Tokyo). The epididymis was divided into three segment; head, body and tail. The epididymal tail was trimmed and minced in 2 ml ham's F10 medium (sigma co) for 30 min, 5% Co_2, 37°C in Co_2 culture device (Model LEEC, England). Sperms with stained cytoplasm in head, neck and tail pieces were considered as death sperms. For this regard eosin-*negrosin* staining technique was conducted; moreover the sperms with any cytoplasmic droplets in mentioned pieces were marked as immature sperms [18].

Total Epididymal Sperm Count and Motility: The cauda epididymis sperm reserves were determined using the standard hemocytometric method and sperm motility was analyzed with microscope (Olympus IX70) at 10 fields and reported as mean of motile sperm according to WHO method [19].

Tubular Differentiation Index (TDI) Determination: To estimate the TDI, the percentage of the STs that were showing more than three layers of differentiated germinal cells from spermatigonia type A, the sections (6µm) were prepared and the STs which showed more than three layers considered as TDI positive [1].

Repopulation Index (RI) Calculation: To determine the RI, the ratio of active spermatogonia (spermatogonia type B with light nucleus in Iron-Weigert staining technique) to inactive spermatogonia (spermatogonia type A with dark nucleus in Iron-Weigert staining technique), in STs was calculated in sections [1].

Serum Sampling and Hormonal Analysis: After days 24,52 and 60, the blood samples from corresponding animals were collected directly from the heart and the serum samples separated by centrifugation. The collected serum samples were subjected to hormonal analysis. The principle of testosterone level measurement in serum was conducted with Radioimmunoassay method.

Statistical Analyses: Statistical analyses was performed on all data using the paired *t*-test to compare quantitative parameters referring to paired organs within a group and two-way analyses followed by a Bonferroni test, using Graph Pad Prism 4.00, Graph Pad Software. P=0.05 was considered as significant difference. All values were expressed as the mean± SD. P= 0.05 was considered to be statistically significant.

RESULTS

Clinical Findings: DZN administration reduced food and water consumption of group two of rats. At the end of treatment period, it came clear that exposing of rats to DZN alone, exerted significant effect on body weight gain (BWG), while co-administration of DZN and SE leaded to better BWG in comparison to DZN alone received animals results (Table 1). Corn oil did not exert any significant effect on BWG in control-sham group. By the time the testicles were decreased in size and weight in DZN consumed rats. Interestingly, SE at studied dose was able to prevent the DZN-induced testicular weight reduction significantly (P<0.05). Furthermore all animals in the DZN-exposed group were observed with decreased movement, staggering gait, occasional trembling and diarrhea.

Histopathological Examination: Histological investigations demonstrated that the rats which received DZN at dose of 300 mg /kg, b.w., orally, once a day for 60 days. showed highly degenerated testes with remarkable atrophy and edema in seminiferous tubules (STs) and interstitial connective tissue as well, while SE considerably lowered the atrophy and edema.

The leydig cells were observed with granulated and hypertrophied cytoplasm in DZN-induced cases. This situation was advanced by the time in all DZN-induced animals. We failed to show any hypertrophied leydig cells in SE applied form of treatment (Figure 1 and 2).

Observations demonstrated that following DZN administration remarkable decrease occurs in germinal epithelium layers number (lower than 3 layers) and more than 50% of the STs were manifested with negative TDI index. In contrast SE+DZN received cases showed higher germinal epithelium (approximately more than 4 layers) with positive TDI index. More analyses with Iron-Weigert staining technique revealed a negative RI which characterized with an increased percentage of spermayogonia type B (active cells) to type A (inactive cells) in DZN-exposed rats. Co-administration of SE+DZN reversed this ratio.

Table 1: The effect of SE on DZN induced changes in body weight gain (BWG) and the ratio of body weight to testicular weight (BW/TW). All data are presented in Mean±SD

BWG (gr) Parameters	24 Days	52 Days	60 Days
Control-sham	199.01±2.00	199.01±2.00	199.01±2.00
DZN	189.00±7.51*	164.20±5.11^{*a}	154.60±3.13^{*b}
DZN+SE	189.80±2.16*	175.80±3.49$^{*a'}$	160.80±2.16$^{*b'}$
(BWG/TW)			
Parameters	24Days	52 Days	60 Days
Control-sham	45.40±1.67	45.40±1.67	45.40±1.67
DZN	46.80±3.96	56.60±2.70^{*c}	66.00±3.93^{*d}
DZN+SE	43.00±3.24	51.60±1.51$^{*c'}$	57.00±1.73$^{*d'}$

Stars show significant differences (P=0.05) between DZN and DZN+SE groups with control-sham and different superscript letters indicate significant differences (P=0.05) between DZN groups with SE+DZN animals at the same column

Table 2: Protective effect of SE on DZN induced negative TDI, RI and seminiferous depletion. All data are presented in Mean±SD

	Seminiferous tubules with negative TDI (%)		
Parameters	24 Days	52Days	60 Days
Control-sham	4.14±0.89	4.14±0.89	4.14±0.89
DZN	7.85±0.89^{*a}	20.42±2.93^{*b}	23.28±1.60^{*c}
DZN+SE	5.57±0.97$^{*a'}$	11.00±1.15$^{*b'}$	15.00±1.91$^{*c'}$
	Seminiferous tubules with negative RI (%)		
	24Days	52 Days	60 Days
Control-sham	1.14±0.69	1.14±0.69	1.14±0.69
DZN	7.08±0.62^{*d}	17.42±2.63^{*e}	30.00±1.29^{*f}
DZN+SE	5.14±0.68$^{*d'}$	11.28±1.11$^{*e'}$	21.42±1.71$^{*f'}$
	Depleted seminiferous tubules (%)		
	24 Days	52 Days	60 Days
Control-sham	0.92±0.73	0.92±0.73	0.92±0.73
DZN	4.00±0.81^{*g}	11.00±1.15^{*h}	19.14±2.03^{*I}
DZN+SE	2.71±0.75$^{*g'}$	9.00±1.00$^{*h'}$	12.28±0.95$^{*I'}$

Stars show significant differences (P=0.05) between DZN and DZN+SE groups with control-sham and different superscript letters indicate significant differences (P=0.05) between DZN groups with SE+DZN animals at the same column.

The germinal epithelium dissociation from tubular lamina properia was detected in more than 30% of STs after 60 days DZN administration while SE could be able to prevent cellular dissociation in SE+DZN groups. More over the rate of STs depletion was progressed by the time in all DZN received rats, but the rats which were treated with SE showed better results after 50 days. After 60 days giant cells were revealed in 4.85±0.50% of the STs in DZN-exposed cases, representing the elimination of atrophied germinal cells residues, Interestingly in SE received animals giant cells were observed rarely (Table 2). DZN-exposed rats demonstrated with high mononuclear immune cells infiltration in the interstitial connective tissue of the testes, which was decreased in SE+DZN received rats (Figure 3).

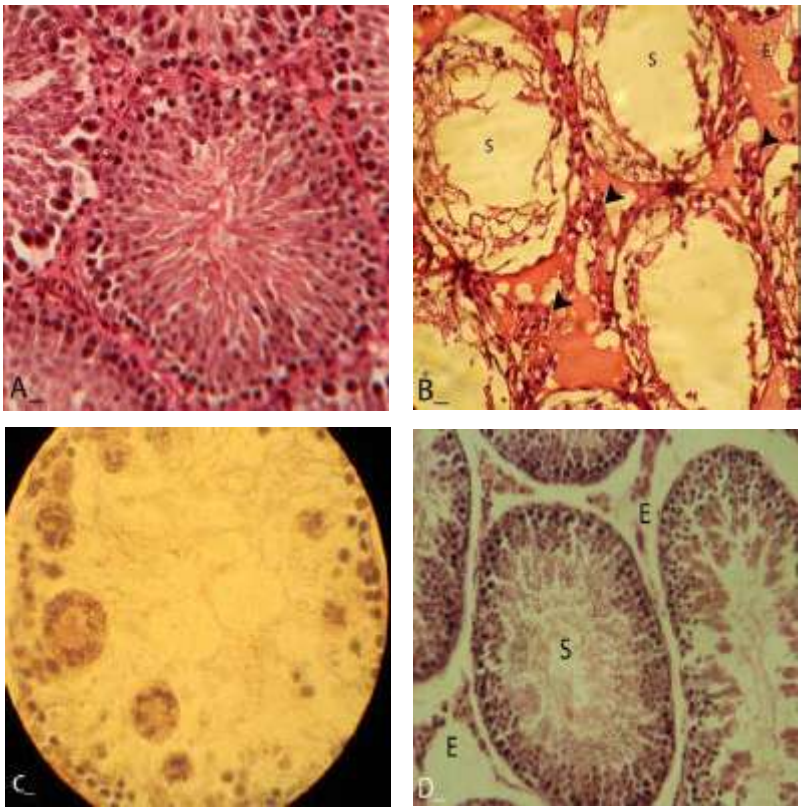

Fig. 1: Cross section from testis; (A) Control-sham group; Note TDI positive seminiferous tubules (germinal epithelium with more than four layers) with no edema in interstitial connective tissue and normal immune cells infiltration. (B) DZN Group; Seminiferous depletion (negative TDI) (S) with sever edema in the interstitial connective tissue. Huge immune cells infiltrations are showed with arrow heads. (C) DZN group; Giant cells appearance in depleted seminiferous tubule. (D) SE+SZN administrated group; Note germinal epithelium of the seminiferous tubules (S) which are presented with higher height in comparison to DZN alone group. Considerable lower edema (E) in the interstitial connective tissue with no giant cells is observable. *Iron-Weigert staining technique (A, B and D: 100X; C: 400X).*

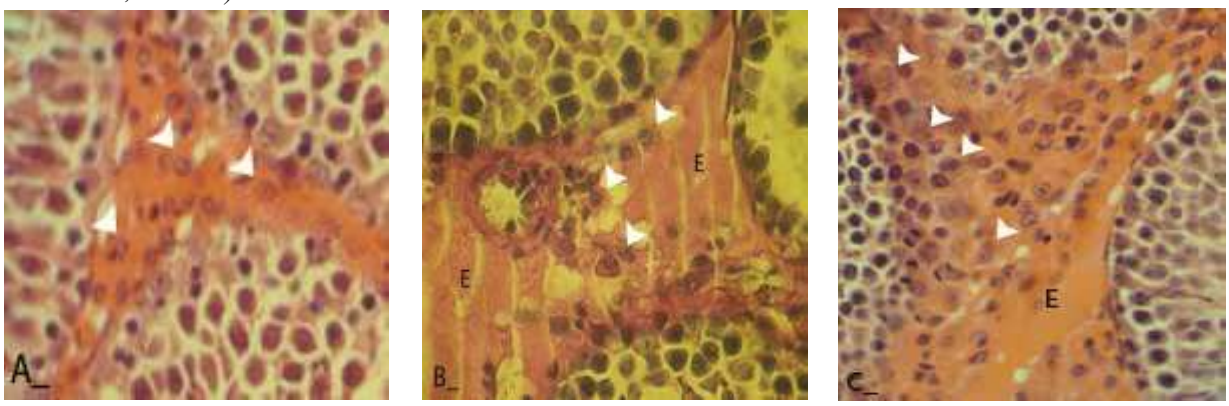

Fig. 2: Cross section from testes; (A) control-sham group; Note leydig cells (Head Arrows) with no hypertrophy, extracted normally in the interstitial connective tissue and are normal in number per one mm^2. (B) DZN alone administrated group; Hypertrophied leydig cells (Head Arrows) which are severely decreased in number in one mm^2 of the connective tissue. Sever edema (E) is detectable in inter tubular region. (C) SE+DZN group; Leydig cells (Head Arrows) are presented approximately with normal histological appearance and these cells are normal in number per one mm^2 of the lower edematic connective tissue. *Iron-Weigert staining technique (400X).*

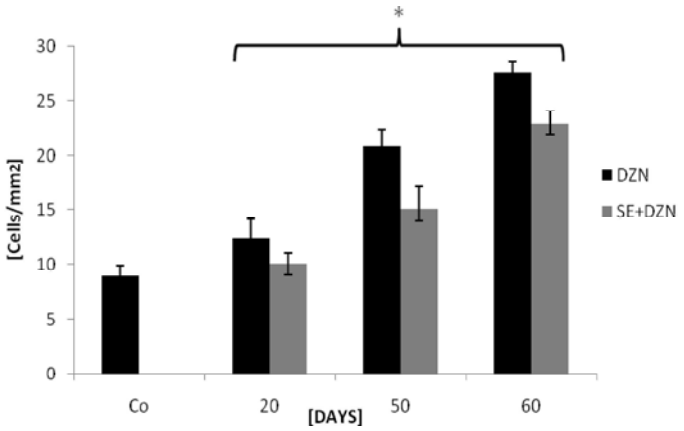

Fig. 3: Mononuclear Immune cells infiltration increased in different test groups. There are significant differences (P=0.05) between all test groups with each other and as well with control-sham group (*). All data are presented in Mean±SD.

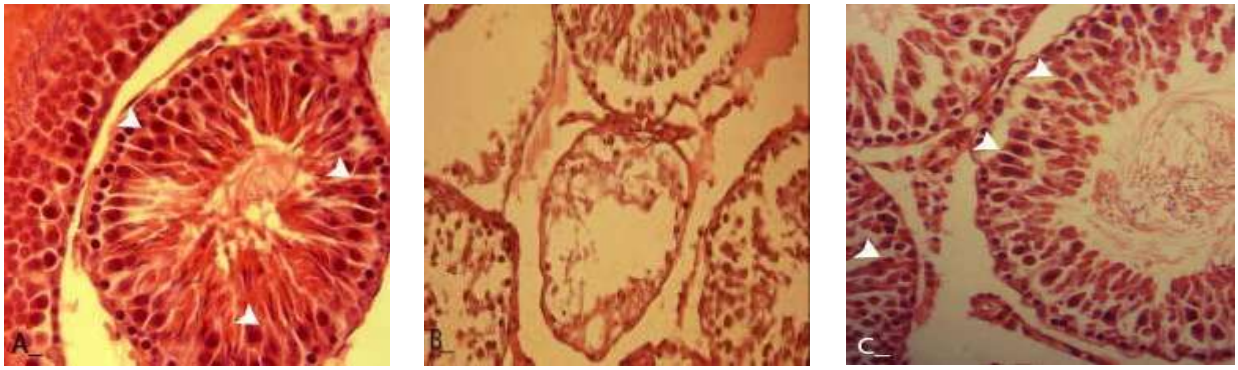

Fig. 4: Cross section from testis; (A) control-sham group; Note spermatogenesis cell series with powerful reaction for PAS staining which are indicating high cytoplasmic carbohydrate supplement while same cells in DZN alone administrated group (B) remained unstained. (C) SE+DZN group; Note the spermatogenesis cell series which are faintly stained with PAS indicating lower cytoplasmic carbohydrate ratio in comparison to control-sham group and higher cytoplasmic supplement in comparison to DZN alone received animals. *PAS staining technique (400X).*

SE Showed Protective Effect on DZN Induced STs Fibrotic Membrane Thickness and Carbohydrate Ratio Reduction in Germinal Cells Cytoplasm: In order to assay the carbohydrates alteration in the testes, PAS staining was conducted on the specimens. Observations demonstrated that in DZN administrated animals the thickness of STs fibrotic membrane increased remarkably in comparison to DZN+SE and control-sham groups. Moreover PAS stained testes showed that the cells in germinal epithelium presented faint reaction to PAS in spermatocytogenesis series (first three layers) while the cells in spermayogenesis process (second three and/or four layers) manifested with dense PAS positive sites. In SE+DZN groups this impairment was moderated to much more powerful response for PAS in spermatocytogenesis cells cytoplasm and fainter PAS reaction in spermayogenesis cell series. Control-sham cases showed dense stained cytoplasm in spermatocytogenesis cells group and very week reactivated cytoplasm in spermayogenesis cells series.

In DZN-induced rats the sertoli cells were manifested with low PAS reactivated sites in their cytoplasm in comparison to DZN+SE and control-sham animals. Similar to sertoli cells in DZN-exposed animals, the spermatozoa which were adjacent to the sertoli cells were revealed with lower reactivated sites and carbohydrate contaminant in their cytoplasm. This situation was near to normal in SE-treated group (Figure 4).

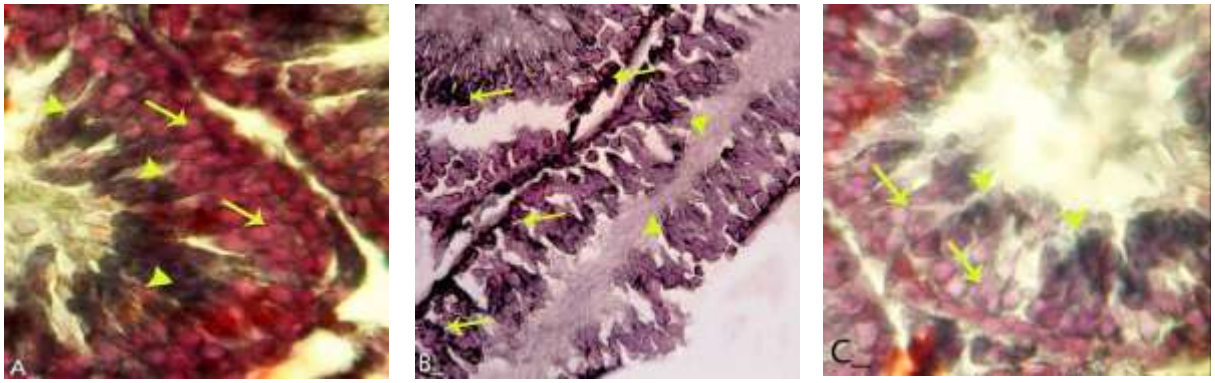

Fig. 5: Cross section from testes; (A) Control-sham group; Note spermatocytogenesis and spermatogenesis cells series with unstained cytoplasms showing that these cells accumulate carbohydrate as a normal source of energy (Arrows) and spermayogenesis series with dark stained cytoplasms (Head Arrows) indicating normal lipid foci accumulation in these cells as an appropriate source of energy for this colony. In contrast to control-sham group in DZN alone group (B) same cells (Arrows) are stained darkly with lipid staining technique indicating that these cells use lipid as an alternative source of energy. Although spermayogenesis cell series (Head Arrows) cytoplasm are stained dark black, steel these cells are stained fainter in comparison to control-sham animals same cell colonies. (C) SE+DZN group; Both spermatogenesis and spermayogenesis cell series are presented approximately normal. *Sudan Black B staining (400X).*

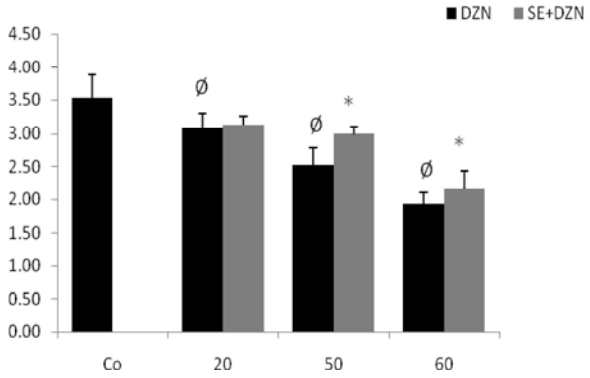

Fig. 6: Mean average of serum testosterone level decreased in different test groups in comparison to control-sham group. There are significant differences (P=0.05) between all test groups with control-sham animals. Ø are indicating remarkable differences (P=0.05) between data in different DZN alone groups (after 20, 50 and 60 days) with each other. Stars are presenting considerable differences (P=0.05) between serum levels of testosterone after 50 days with data after 60 days in SE+DZN groups. All data are presented in Mean±SD.

SE Exerted Protective Effect on DZN Induced Lipid Accumulation in the Cytoplasm of the Germinal Cells: Histological analyses showed high lipid accumulation in the cytoplasm of the spermatocytogenesis series of longtime DZN-administrated rats. The lipid foci ratio was diminished in the spermatozoa content of the STs in DZN-induced animals. Mean while the SE-treated animals were observed with approximately normal lipid reactivated sites in the spermatozoa contents and much lower cytoplasmic lipid accumulation in first three cell layers of the germinal epithelium (Figure 5).

SE Increased the DZN Reduced Testosterone Level in Blood Circulation: The serum analyses showed that the chronic exposing to DZN could be able to decline the testosterone level significantly (P=0.05). Accordingly by the time the animals which were dosed with DZN alone were revealed with decreased testosterone. Co-administrated animals with SE+DZN were demonstrated with higher testosterone level in comparison to the DZN alone dosed animals. No serological alterations were occurred in testosterone level of control-sham rats (Figure 6).

SE Improved DZN Induced Sperm Abnormalities, Immaturities and Mortality: Light microscopic investigations using eosin-negrosin staining revealed increased abnormal and immature sperm ratio with elevated sperm mortality in the DZN alone administrated rats. This impairment developed by the time.

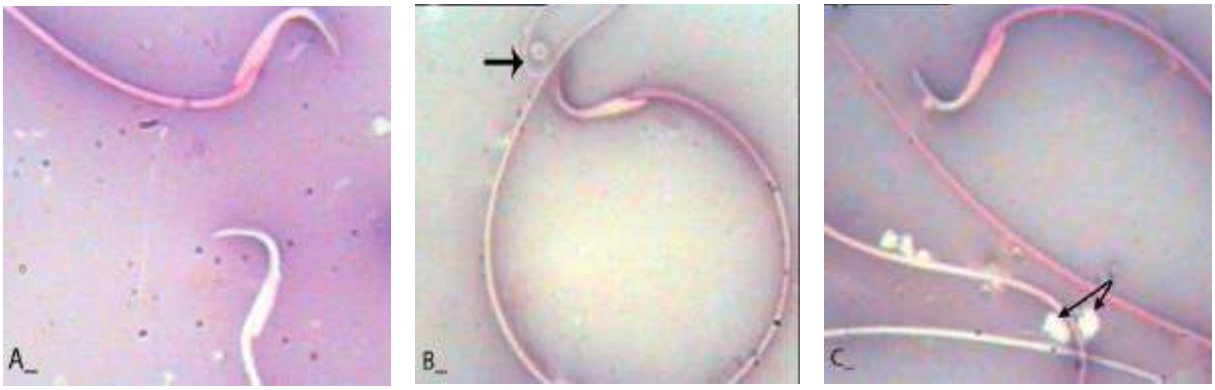

Fig. 7: Light microscopic architecture from sperms; (A) Death (sperm with stained head) and normal sperms (unstained sperm). (B) Death sperm with abnormal tail (Arrow). (C) Abnormal sperm with cytoplasmic droplet (Arrows). *Eosin negrosin staining technique (1000X).*

Table 3: Protective effect of SE on DZN induced impact on sperm volume, motility, maturity, DNA integrity and mortality. All data are presented in Mean±SD

	Sperm count (NO×10^6)		
Parameters	24 Days	52 Days	60 Days
Control-sham	54.00± 2.03	57.3± 2.87	51.08± 1.86
DZN	32.33± 1.21^{*f}	33.10± 1.43	25.00± 1.09^{*f}
DZN+SE	35.97± 1.55$^{*f'}$	38.04±1.73$^{*f'}$	36.00± 1.66
	Sperm motility (%)		
	24 Days	52 Days	60 Days
Control-sham	89.25±1.83	89.25±1.83	89.25±1.83
DZN	82.00±1.69^{*a}	55.62±2.87^{*b}	47.12±2.58^{*c}
DZN+SE	83.37±2.13$^{*a'}$	64.00±3.46$^{*b'}$	49.59±3.89$^{*c'}$
	Sperm Immaturity (%)		
	24 Days	52 Days	60 Days
Control-sham	12.71±1.60	12.71±1.60	12.71±1.60
DZN	17.71±1.79^{*d}	25.42±1.72^{*e}	33.00±2.94^{*f}
DZN+SE	14.00±2.16$^{*d'}$	19.57±1.51$^{*e'}$	24.71±2.56$^{*f'}$
	Sperm DNA disintegrate (%)		
Control-sham	9.37±1.06	9.37±1.06	9.37±1.06
DZN	20.00±1.77^{*g}	27.37±1.59^{*h}	33.00±2.87^{*I}
DZN+SE	15.00±2.13$^{*g'}$	21.62±2.26$^{*h'}$	30.00±1.30$^{*I'}$
	Sperm mortality (%)		
	24 Days	52 Days	60 Days
Control-sham	16.37±1.30	16.37±1.30	16.37±1.30
DZN	21.87±1.80^{*J}	37.62±1.68^{*k}	49.50±1.60^{*L}
DZN+SE	18.00±1.06$^{*J'}$	33.5±2.67$^{*k'}$	46.12±1.88$^{*L'}$

Stars show significant differences (P=0.05) between DZN and DZN+SE groups with control-sham and different superscript letters indicate significant differences (P=0.05) between DZN groups with SE+DZN animals at the same column.

Interestingly the animals witch received SE+DZN were manifested with lower abnormal, death, immature sperm volume and increased motility (Figure 7). According to our sperm count after long time dosing of DZN, the rats were demonstrated with very low sperm volume. Comparing sperm number in DZN alone and DZN+SE received rats revealed that the SE consumed animals exhibit higher sperm number. No histopathological changes were identified in control-sham group sperm evaluations. The data for sperm parameters are presented in table 3.

DISCUSSION

DZN is one of the well known contaminants which often found in human and animal foods and in less extent in consumed water. Several factors including dose, route of exposure, percent of absorption, physicochemical property and rate of detoxification play essential roles in severity of poisoning. Mild poisonings are seen after accidental exposure by the dermal and pulmonary routes; but, in severe poisonings, these agents have been used for suicidal purpose by oral ingestion [17]. Due to huge impact of DZN on humans and animals health, it seems still investigation about its effect on various organs would be worthwhile. The purpose of the current study was to uncover any pathological impact of relatively chronic exposure against DZN in male reproductive system in particular on testes and consequently investigate the potential protective effect of SE on DZN-induced damages.

In the first part of this study we aimed to show how DZN-exposure could exert pathological impact on the testes tissue in detail. To reach this goal we used various

well known histopathological approaches to make clear all alterations. Very early and considerable finding of first step was that, DZN induces sever degeneration in STs. In contrast the SE can prevent the adverse effects of DZN and as well can improve DZN detrimental effects, accordingly the rats which were received SE revealed with histologically recovered STs.

Another important histopathological finding of this study is that following DZN-exposure, we found in germinal epithelium, simultaneously dissociation of germinal cells and arrested spermatocytogenesis, spermatogenesis and spermayogenesis with sever depletion of STs which accompanied with huge infiltration of inflammatory cells. All mentioned pathological signs are representing an inflammation resulted from DZN exposure. There are several independent reports which are indicating that following sever inflammation, elevated oxidative stress causes apoptosis in spermatogenesis series and thus the remarkable cellular depletion occurs in STs after acute apoptosis [23-24]. In confirming of these reports our histological findings showed that DZN is capable to induce structural and functional damages (considerable apoptosis, STs depilation) in testes and consequently during DZN induced inflammation the permeability of blood vessels increases and leads to sever edema [25-30]. Identification of negative RI index in DZN administrated animals helped us to come close to this fact that spermatogonia cells are very susceptible to DZN and this compound can arrest the repopulation ratio in STs and in turn it can lead to significant reduction in active spermatogonia cells population. Therefore by this pathological mechanism spermatocytogenesis arrest occurs in most of the STs. Instead in SE-administrated animals the histopathological analyses were showed that the rate of inflammatory reaction was declined.

According to previous reports the glucose is the main source of energy for spermatogenesis cells series and/or entirely for three first layers of germinal epithelium, in order to synthesizing essential proteins and also biological activities [33]. Since glucose transporters are the main transporting elements to transferring glucose through STs hyper-mitotic cell series [34], any degeneration event made by DZN could result to interruption in glucose metabolism. Thus in order to identify cytoplasmic carbohydrate ratio in mentioned cell series (following DZN administration), PAS staining technique was conducted. Histochemical analyses showed that in DZN alone groups, the first three layers of germinal cells exhibited faint cytoplasmic carbohydrate accumulation. In contrast SE received animals were manifested with significantly higher cytoplasmic carbohydrate supplement. This situation suggests that SE was able to exert protective effect on DZN induced reduction in carbohydrate ratio by performing approximately normal glucose transportation to STs. Therefore the germinal cells were preserved their ability to use carbohydrate sources (mainly glucose) in order to save adequate energy.

This situation suggested us to think about another hypothesis; in the case of missing energy sources in DZN induce cases the spermatogonia cells and spermatogenesis cell series switched their energy sources from glucose to other elements. Lipids could be the second and/or best choice for saving energy in those cells. Therefore in order to identify these probable changes we used Sudan Black staining and observations demonstrated that in contrast to SE received groups in DZN alone animals first three layer of germinal epithelium exhibited with dense cytoplasmic lipid foci acculmulation. This impairment advanced by the time. These findings can help us to come close to this fact that although oxidative stress could be able to exert apoptosis following sever inflammation as a primary phase, inadequate energy supplement in hyper-mitotic cells was continued ruining effects of DZN in DZN alone groups.

On the other hand the importance of androgens for normal spermatogenesis is well documented lastly. Previous studies indicated that most of the OP compounds inhibit the non-specific esterase activities in Leydig cells, that in turn can result in reduced testosterone production [27,28]. Testosterone, through different pathways, can affect Sertoli cells physiological function [29,30]. Any functional damages in sertoli cells can lead to germinal cells degeneration and disruption. In the light of these pathophysiological pathways our histological investigations demonstrated that the leydig cells decreased in number / mm^2 of the interstitial connective tissue of the DZN groups. Moreover our hormonal analyses showed the testosterone reduction in DZN dosed animals. The normal spermatogenesis processes depend on testosterone production by leydig cells in response to FSH and LH hormones [35-37]. Therefore, it would be more logic to hypothesize that DZN-exposure resulted in leydig cells degeneration, leaded to reduction in blood testosterone level and consequently caused sertoli cells dysfunction. Ultimately sertoli cells dysfunction in turn could be able to result in germinal cells degeneration and dissociation in STs of DZN animals. In contrast the animals which were SE-administered showed higher serum testosterone level and

therefore much more leydig cells in one mm^2 of the interstitial tissue. Thus these animals were manifested with better germinal cells junctions in histological analyses. Another feature for DZN induced degenerative impact which was confirming previous reports was Apoptotic degeneration of early spermatogenic cells is proposed to be a mechanism regulating the quantity and quality of sperm produced in mammalian testis [38].

Lastly it is well proofed that the semen oxidative changes have been associated with apoptosis in sperm contents [39]. The main and primary source of high Reactive oxygen species production is the immature spermatozoa having residual cytoplasm and DNA damages of sperm contents [40-43]. There are plenty of evidences in the present study including; increased abnormal, death, immotile and immature sperm number in DZN-induced animals witch are indicating the probable major role of imbalanced oxidative stress condition in generating of various disorders.

CONCLUSION

These findings can suggest us to conclude that the direct effects of the DZN induced sever apoptosis in the germinal cells and remarkable germinal cells degeneration lowered the sperm quality and quantity. Therefore high content of pressured germinal cells, elevated abnormal, immature, death sperms and high infiltration of immune cells.

ACKNOWLEDEGMENT

The authors wish to thank department of comparative histology and embryology, Faculty of veterinary medicine, Urmia university.

REFERENCES

1. Sarabia, L., I. Maurer and E. Bustos-Obregón, 2009. Melatonin prevents damage elicited by the organophosphorous pesticide diazinon on the mouse testis. Ecotoxicology and Environmental Safety, 72: 8-942
2. Poet, T.S., AA. Kousba, S.L. Dennison and C. Timchalk, 2004. Physiologically base pharmacokinetic/pharmacodynamic model for the organophosphorus pesticide diazinon. Neurotoxicology, 25: 1013-1030.
3. Fattahi, E., K. Parivar, S.G.A. Jorsaraei and A.A. Moghadamnia, 2009. Iranian Journal of Reproductive Medicine, 7: 59-64.
4. Slotkin, T.A. and F.J. Seidler, 2007. Comparative developmental neurotoxicity of organophosphates *in vivo*: Transcriptional Responses of pathways for brain cell development, cell signaling, cytotoxicity and neurotransmitter systems. Brain Research Bulletin, 30: 232-27.
5. Narayana, K.N., L.D. Prashanthi and U. Souza, 2006. An organophosphate insecticide methyl parathion (0-0-dimethyl0-4-nitrophenyl phosphorothioate) induces cytotoxic damage and tubular atrophy in the testis despite elevated testosterone level in rats. Journal of Toxicology Science, 31: 177-189.
6. Hatjian, B.A., E. Mutch, F.M. Williams, P.G. Blain and J.W. Edwards, 2000. Cytogenetic response without changes in peripheral cholinesterase enzymes following exposure to a sheep dip containing diazinon *in vivo* and *in vitro*. Mutation Research, 427: 85-92.
7. Aluigi, M.G., C. Angelini, C. Falugi, R. Fossa, P. Genever, L. Gallus, *et al.,* 2005. Interaction between organophosphate compounds and cholinergic functions during development. Chemical and Biological Interactions, 157: 305-316.
8. Munshi, C.B., G.F. Combs and N.I. Mondy, 1990. Effects of selenium on the nitrogenous constituents of the potato. Jounal of Agricultur and Food Chemistry, 38: 2000-2002.
9. Ip, C., D.J. Lisk and H.E. Ganther, 2000. Chemoprevention with triphenylselenonium chloride in selenium-deficient rats. Anticancer Research, 20: 4179-4182.
10. Miller, S., S.W. Walker, J.R. Arthur, F. Nicol, K. Pickard, *et al.,* 2001. Selenite protects human endothelial cells from oxidative damage and induces thioredoxin reductase. Clincal Science, 100: 543-550.
11. Nava, M., F. Romero, Y. Quiroz, G. Parra, L. Bonet and B. Rodríguez-Iturbe, 2000. Melatonin attenuates acute renal failure and oxidative stress induced by mercuric chloride in rats. American Journal of Renal Physiology, 279: 910-918.
12. Mayerly, N., R. Freddy, Q. Yasmir, P. Gustavo, B. Lizette and I. Bernardo Rodríguez, 2000. Melatonin attenuates acute renal failure and oxidative stress induced by mercuric chloride in rats. American Journal of Renal Physiology, 279: 910-918.
13. Bansal, A.K. and G.S. Bilaspuri, 2009. Antioxidant effect of vitamin E on motility, viability and lipid peroxidation of cattle spermatozoa under oxidative stress. Animal Science Papers and Reports, 27: 5-14.

14. Aziz, N., S.W. Shah and R.A. Aziz, 2008. Histological Changes in Male Rat Reproductive Organs Post-Treated With Insecticide Carbofuran (Furadan) Annals Microscopy, 8: 83-89.
15. Banerjee, B.D., V. Seth, A. Battacharya, S.T. Pasha and A.K. Chakrabory, 1999. Biochemical effects of some pesticides on lipid peroxidation and free radical scavengers. Toxicology Letters, 107: 33-47.
16. Etemadi-Aleagha, A., M. Akhgari and M. Abdollahi, 2002. A brief review on oxidative stress and cardiac diseases. Middle East Pharmacology, 10: 8-9.
17. Suzuli, N. And N. Sofikitis, 1999. Protective Effects of Antioxidants on Testicular Functions of Varicocelized Rats. Yonago Acta medica, 42: 87-94.
18. Matthews, G.J., E.D. Matthews and M. Goldstein, 1998. Induction of spermatogenesis and achievement of pregnancy after microsurgical varicocelectomy in men with azoospermia and severe oligoasthenospermia. Fertility and Sterility, 70: 71-75.
19. Esteves, S.C. and S. Glina, 1999. Recovery of spermatogenesis after microsurgical varicocele repair in azoospermic men is related to testicular histology. Jounal of Urology, 161: 1199-311.
20. Brinkworth, M.H., G.F. Weinbauer and M. Bergman, 1997. Apoptosis as a mechanism of germ cells loss in elderly men. International Journal of Andrology, 20: 222-228.
21. Marwar, J.L., 2001. The pathophysiology of varicocele in light of current molecular and genetic information. Human Reproduction, 7: 461-472.
22. Lue, Y.H., A.P. Sininhltilcim and R.S. Swerdloff, 1999. Single exposure to heat induces stage specific germ cell apoptosis in rat; role of intratesticular testosterone on stage specifity, Endocrinology, 140: 1709-1717.
23. Fujishiro, J.A., 1995. Effect of varicocele on fertility potential: comparison between impregnating and non-impregnating groups. Archive of Andrology, 35: 143-148.
24. Wesseling, C., D. Antich, C. Hogstedt, A.C. Rodríguez and A. Ahlbom, 1999. Geographical differences of cancer incidence in Costa Rica in relation to environmental and occupational pesticide exposure. International Journal of Epidemiology, 28: 365-374.
25. Pidoux, G., P. Gerbaud, V. Tsatsaris, O. Marpeau, F. Ferreira, G. Meduri, *et al.,* 2007. Biochemical characterization and modulation of LH/CG-receptor during human trophoblast differentiation. Jounal of Cell Physiology, 212: 26-35.
26. Hess, R.A. and M. Nakai, 2000. Histopathology of the male reproductive system induced by the fungicide benomyl. Histology and Histopathology, 15: 207-224.
27. Boekelheide, K. and H.A. Schoenfeld, 2001. Spermatogenesis by Sisyphus: proliferating stem germ cells fail to repopulate the testis after 'irreversible' injury. Advances in Experimental Medicine and Biology, 500: 421-428.
28. Izumi, H., E. Kimura, T. Ota and S. Shimazu, 2005. A two-generation reproductive toxicity study of n-butylbenzene in rats. Journal of Toxicology Science, 30: 21-38.
29. Gomez, E., D.S. Irvine and R.J. Aitken, 1998. Evaluation of a spectrophotometric assay for the measurement of malondialdehyde and 4-hydroxyalkenals in human spermatozoa: relationships with semen quality and sperm function. International Journal of Andrology, 21: 81-94.
30. Farooqi, I.S. and S.O. Rahilly, 2004. Monogenic Human Obesity Syndromes. Recent Progress in Hormone Research, 59: 409-424.
31. Bustos-Obregón, E. and P. González-Hormazabal, 2003. Effect of a single dose of malathion on spermatogenesis in mice. Asian Journal of Andrology, 5: 105-107.
32. Chapin, R.E., J.L. Phelps, S.G. Somkuti, J.J. Heindel, L.T. Burka, 1990. The interaction of Sertoli and Leydig cells in the testicular toxicity of tri-o-cresyl phosphate. Toxicology Applied Pharmacology, 104: 483-95.
33. Skinner, M.K. and I.B. Fritz, 1985. Testicular peritubular cells secrete a protein under androgen control that modulates Sertoli cell functions. Proceeding of the National Academy of Science, 82: 114-8.
34. Kackar, R., M.K. Srivastava and R.B. Raizada, 1993. Assessment of toxicological effects of mancozeb in male rats after chronic exposure. Indian Journal of Experimental Biology, 37: 553-559.
35. Kerr, J.B., R.A. Mayberry and D.C. Irby, 1984. Morphometric studies on lipid inclusions in Sertoli cells during the spermatogenic cycle in the rat, Cell Tissue Research, 236: 699-709.
36. Hatjian, B.A., E. Mutch, F.M. Williams, P.G. Blain and J.W. Edwards, 2000. Cytogenetic response without changes in peripheral cholinesterase enzymes following exposure to a sheep dip containing diazinon *in vivo* and *in vitro*. Mutatation Research, 427: 85-92.

37. Prashanthi, N., K. Narayana, A. Nayanatara, H.H. Chandra Kumar, K.L. Bairy, *et al.,* 2006. The reproductive toxicity of the organophosphate pesticide 0, 0-dimethyl 0-4-nitrophenyl phosphorothioate (methyl parathion) in the male rat. Folia Morphology, 65: 309-321.
38. Alessio, D., A. Riccioli, P. Lauretti, F. Padula, B. Muciaccia, P. De Cesaris, *et al.,* 2001. Testicular FasL is expressed by sperm cells. Proceeding of the Nationall Academy of Science, 98: 3316-3321.
39. Dalsenter, P.R., A.S. Faqi, J. Webb, H.J. Merker and I. Chahoud, 1996. Reproductive toxicity and tissue concentrations of lindane in adult male rats, Human Experimental Toxicology, 15: 406-410.
40. Khayatnouri, M. And S.E. Safavi, 2008. The effect of Saffron Orally Administration on Spermatogenesis Index in Rat. Advances in Environmental Biology, 5(7): 1514-1521, 2011.