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Protective Role of Selenium Against Adverse Effects of Atrazine Toxicity in Male Rats: Biochemical, Histopathological and Molecular Changes

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Abstract: The present study was conducted to evaluate the protective effect of selenium against atrazine-induced oxidative damage in experimental male rats. 40 male rats were divided into four equal groups; the first was served as control and the remaining groups were treated with atrazine (200 mg/kg body weight), selenium (300 µg/kg body weight) and a combination of atrazine and selenium, respectively. Plasma samples were examined for changes in liver enzyme activities, testosterone and estradiol concentrations as well as malondialdehyde (MDA) as indicator of lipid peroxidation and superoxide dismutase (SOD) and total antioxidant capacity (TAC) as antioxidant markers. Testes and prostate gland tissue samples were examined for histopathological changes. In addition, genomic DNA fragmentation was performed to hepatic tissues. Obtained results revealed that exposure of rats to atrazine induced a significant increase in plasma MDA and SOD as well as activities of AST and ALT. A significant decrease in testosterone levels was observed in atrazine-treated group with non-significant changes in estradiol levels. In group treated with selenium and atrazine, an elevated blood testosterone levels was noticed. Histopathological findings of testes of atrazine treated rats showed necrobiotic changes of spermatogonial cells, meanwhile, prostatic acini showed marked hyperplasia of the epithelium lining and cystic dilatation. On the other hand, spermatogenesis in most of seminiferous tubules of rat testes treated with atrazine and selenium were within normal apperance. Atrazine induced sever hepatic DNA fragmentation in atrazine treated group with mild protective effect of selenium in atrazine/ selenium group. In conclusion, results suggest that selenium could protect against atrazine induced adverse effects on liver and testicular functions and has a weak protection against DNA fragmentation in male rats.

Key words: Selenium · Atrazine · Oxidative stress · Testis · DNA fragmentation · Rats

INTRODUCTION

Atrazine, a triazine-based herbicide extensively used in agriculture worldwide, has been reported to induce oxidative stress in several experimental models [1]. The injurious effects of atrazine were related to its generation of reactive oxygen species (ROS) that cause oxidative stress of various organs [2]. However, ROS must be continuously inactivated by antioxidants to keep the oxidant/antioxidant balance to maintain normal cell function [3]. Lipid peroxidation is most often induced by superoxide radical(O_{-2}) and its damage is mainly inhibited by SOD [4]. TAC covers both enzymatic and non-enzymatic antioxidants [5] and its measuring considers the cumulative effect of all antioxidants present in plasma and body fluids [6].

Atrazine oral exposure has adverse effects on the testicular and epididymal sperm numbers, motility, viability, morphology and daily sperm production in rats [7]. Hepatic damage was reported due to exposure to atrazine in rats [8]. Additionally, atrazine exposure was associated with DNA damage and genotoxicity [9]. Atrazine was tested for genotoxicity including assays for DNA damage and repair in bacteria and mammalian cells in vitro [10] and for chromosomal aberrations *in vitro* [11].

Selenium treatment was observed to ameliorate the toxicity induced by several chemicals and drugs [12-14]. Selenium is an integral part of many proteins and its importance in mammalian cell is due to its function in the active site of a number of selenium-dependent enzymes which is known as selenoprotiens [15]. During selenoprotiens synthesis, it is incorporated into a very

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specific location in the amino acid sequence in order to form a functional protein [16]. Selenium functions as a redox center, for instance when the selenoenzyme, thioredoxin reductase, reduces nucleotides in DNA synthesis and helps control the intracellular redox state [17]. The best-known example of this redox function is the reduction of hydrogen peroxide and damaging lipid and phospholipid hydroperoxides to harmless products (water and alcohols) by the family of selenium-dependent glutathione peroxidases [18]. This function helps to maintain membrane integrity, to protect prostacyclin production and to reduce the likelihood of propagation of further oxidative damage to biomolecules such as lipids, lipoproteins and DNA [16, 19]. While data concerning atrazine toxicity in mammals are increasing, studies of the antioxidant potency of selenium against atrazine induced toxicity in rats are still scarce. The present study was designed to evaluate the protective capacity of selenium against atrazine-induced effects on oxidant/antioxidant statue, liver and testicular functions as well as DNA integrity in male rats.

MATERIALS AND METHODS

Animals: A total of 40 adult 10 weeks old laboratory-bred strain Swiss albino male rats with average weight (80 - 120 g) were selected for this study. Animals were obtained from the Animal House Colony of the National Research Centre (Cairo, Egypt). They were kept under standard laboratory conditions. The animals were given free access to food and water.

Experimental Design: One week after acclimatization, animals were randomly divided into 4 equal groups as follows: group 1, acontrol group, which was put on a normal diet without any treatments; group 2, atrazine group, which received commercial atrazine (Technical grade, 80.0% - Oxon - Italy) orally 200 mg/kg body weight [20] 3 times a week for 8 weeks; group 3, selenium group, which received 300µg/kg body weight selenium [21] by oral route 3 times a week for 8 weeks; group 4, atrazine and selenium treated group, this group treated by oral route with 200 mg/kg body weight atrazine and 300 µg /kg selenium for 3 times a week for 8 weeks.

Sampling

Blood sampling: At the end of the experiment, blood samples from the retro orbital venous plexus were received into heparinized tubes and centrifuged at 3000 r.p.m. for 10 minutes and plasma were kept at –20°C till analysis.

Tissue Samples: All rats were sacrificed at the end of the experiment. Tissue samples from testes, prostate gland and liver were collected. Testes and prostate gland were fixed in 10% neutral buffered formalin for histopathological examinations. Parts of liver tissues were washed in distilled water and kept at -80 till be used for DNA extraction to assess the level of DNA fragmentation analysis.

Laboratory Examinations

Biochemical Analysis

Oxidant and Antioxidant Assays: Oxidants and antioxidants biomarkers in plasma were evaluated spectrophotometrically using kits purchased from Biodiagnostic, Egypt:

MDA level was determined according to the method of Ohkawa, *et al*. [22].

TAC was determined as described by Koracevic *et al*. [23].

SOD activity was measured according to the method adopted by Nishikimi, *et al*. [24].

Testosterone and Estradiol Assays: Testosterone and estradiol levels were assayed using kits from DRG, Germany as described by Marcus and Durnford [25] and Cameron and jones [26], respectively.

Some Liver Enzyme Tests: Plasma AST and ALT activities were determined kinetically according toReitman and Frankel [27] using kits purchased from Biodiagnostic, Egypt.

Histopathological Examinations: Tissue samples from testes and prostate gland were fixed in 10% neutral buffered formalin. The specimens were dehydrated, embedded in paraffin, sectioned at 5 μ thickness and stained with haematoxylin and eosin (H&E) and then examined microscopically [28].

DNA Fragmentation Analysis: High quality genomic DNA was extracted from -80°C preserved liver samples of all treated and control groups by precipitation of protein and other contaminants and further precipitation of high molecular weight genomic DNA by absolute ethanol [29]. The extent of DNA fragmentation (DNA ladder) has been assayed by electrophoresing genomic DNA sampleson 1.5% agarose gel stained with ethidium bromide and visualized using UV transilluminator [30].

Statistical Analysis: Data were subjected to statistical analysis including the calculation of the mean and standard error of the mean. One way ANOVA was used and Duncan's Multiple Range test were used to differentiate between significant means [31].

RESULTS

Biochemical Tests

Oxidant/antioxidant Assays: Table (1) indicated that while MDA levels were significantly (P<0.05) higher in atrazine treated rats, their levels weren't altered significantly in both selenium and atrazine/selenium groups in comparison with control one. Moreover, atrazine treated animals had significantly (P<0.05) higher SOD activity when compared to atrazine/ selenium rats which in turn, had elevated activity of SOD in comparison with control group. Although non-significant, TAC was higher in rats given selenium alone. However, TAC levels didn't reveal any significant changes in both atrazine and atrazine/selenium groups in comparison with control one.

Testosterone and Estradiol Assays: It is evident from data presented in Table (2) that levels of testosterone were significantly (P<0.05) decreased in atrazine treated group in comparison with control group. Supplementation of selenium along with atrazine showed a significant (P<0.05) increase in testosterone levels as compared to atrazine treated rats. Moreover, there was non-significant elevation of estradiol concentrations in atrazine treated rats when compared to control group.

Some Liver Enzyme Tests: Data presented in Table (3) revealed that alanine aspartate amino transferase (AST) and alanine amino transferase (ALT) activities were significantly (P<0.05) higher in atrazine treated group when compared to control group. In the same time, the co-administration of selenium has significantly (P<0.05) reduced the activities of AST and ALT with no significant changes in AST and ALT activities in rats given selenium alone.

Table 1: Effect of exposure of male rats to atrazine, selenium and atrazine with selenium on levels of MDA (nmol /ml), TAC (mM/L) and SOD (U/ml):

	Groups			
Biochemical parameters	Control	Atrazine	Selenium	Atrazine/Selenium
MDA (nmol /ml)	10.93^{a}±1.115	13.26^{b} ±2.19	10.98^{a} ±1.578	10.99^{a} ±0.88
TAC (mM/L)	0.17^{a} ±0.05	0.18^{a} ±0.03	0.22^{a} ±0.07	0.19^{a} ±0.03
SOD (U/ml)	322.9^{a}±28.08	631.3^{c}±48.90	387.98^{a}±18.71	519.31^{b} ±35.17

-Data are presented as means ± SE.

-Means having different superscripts within the same raw are significantly (P<0.05) different.

Table 2: Effect of exposure of male rats to atrazine, selenium and atrazine with selenium on levels of testosterone (ng/ml) and estradiol (pg/ml):

	Groups			
Biochemical parameters	Control	Atrazine	Selenium	Atrazine/Selenium
Testosterone (ng/ml)	3.28^{b} ± 0.37	2.34^{a} ± 0.12	3.79^{b} ± 0.38	3.39^{b} ± 0.23
Estradiol (pg/ml)	1.69±0.10	1.93±0.03	1.76±0.09	1.87±0.07

-Data are presented as means ± SE.

-Means having different superscripts within the same raw are significantly (P<0.05) different.

Table 3: Effect of exposure of male rats to atrazine, selenium and atrazine with selenium on activities of AST and ALT (U/L):

	Groups			
Biochemical parameters	Control	Atrazine	Selenium	Atrazine/Selenium
AST (U/L)	84.26^{a} ±8.17	128.61^{b} ±9.89	81.28^{a} ±11.68	93.79^{a} ±12.93
ALT (U/L)	71.50^{a} ±10.5	92.29^{b} ±12.66	68.50^{a} ±10.37	69.63^{a} ±13.70

-Data are presented as means ± SE.

-Means having different superscripts within the same raw are significantly (P<0.05) different.

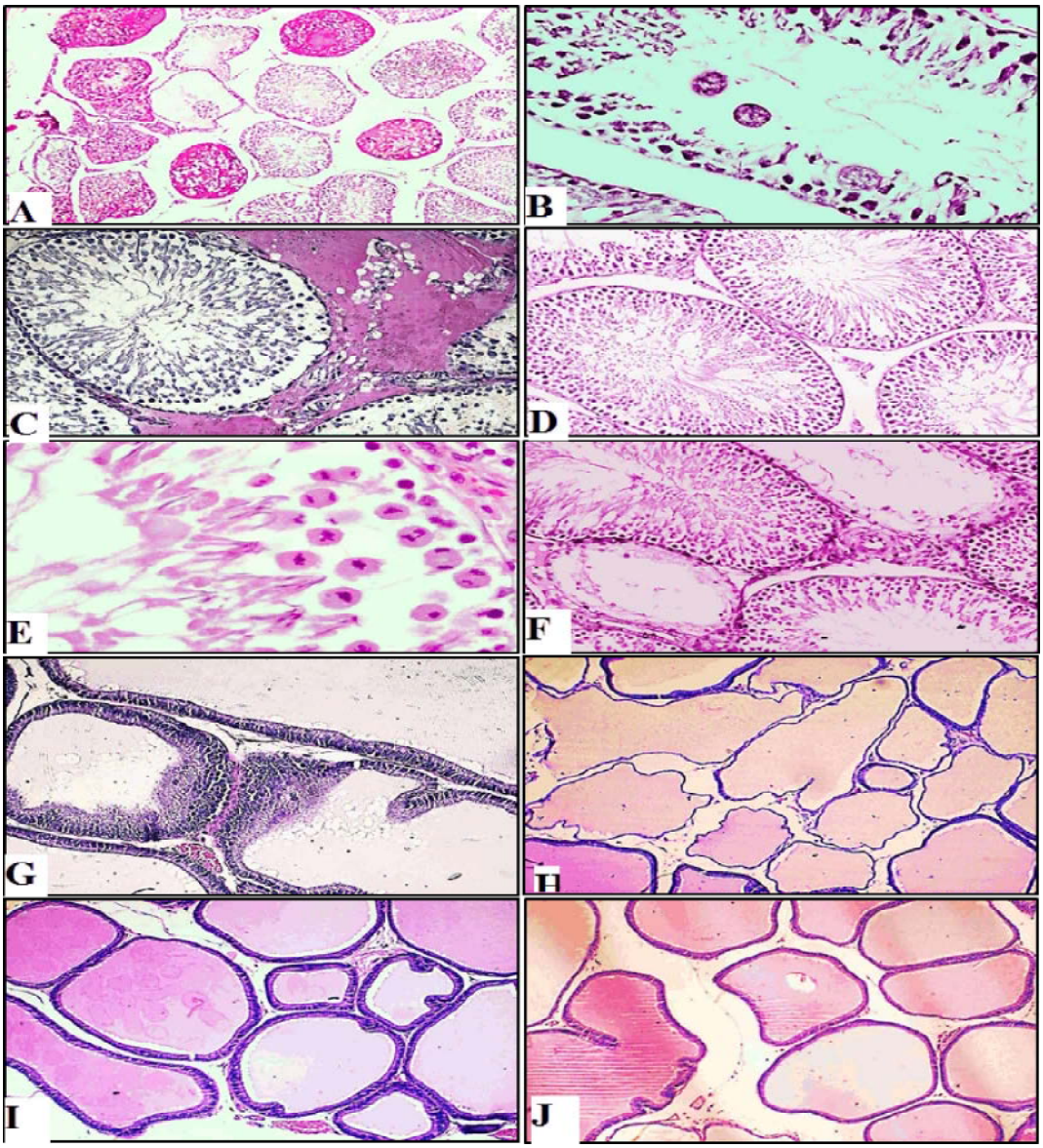

Fig. 1: A.Testis of atrazine treated rats, showing vacuolar degeneration and necrobiotic changes of spermatogonial cell layers of seminiferous tubules, some tubules contain homogenous hyalinized eosinophilic material with no sperms within the lumen (H&E,X100). Fig. (B): Testis of atrazine treated rats, showing multinucleated giant cells and cellular debris within the lumen of some tubules. (H&E, X200). Fig. (C): Testis of atrazine treated rats, showing focal interstitial edema. (H&E, X100). Fig. (D): Testis of selenium treated rats, showing normal spermatogenesis as well as spermatogonial cells (H&E, X100). Fig. (E): Testis of selenium treated rat, showing different stages of mitotic division among the spermatogonial cells (H&E, X200). Fig. (F): Testis of rat treated with atrazine and selenium, showing normal spermatogenesis in some tubules meanwhile, others showing complete depletion of spermatogenic epithelium lining (H&E, X100). Fig. (G): prostate gland of atrazine treated rat, showing marked focal hyperplasia of the epithelial lining of acini (H&E, X100). Fig. (H): prostate gland of atrazine treated rat, showing cystic dilatation of acini associated with irregularity of its outline (H&E, X100). Fig. (I): prostate gland of selenium treated rat, showing normal acini with normal folded basophilic epithelial layer of columnar and/or cuboidal cells (H&E, X100). Fig. (J): prostate gland of rat treated with atrazine and selenium, showing mild hyperplasia of epithelial lining of some of the prostatic acini and cystic dilatation in few acini (H&E, X100).

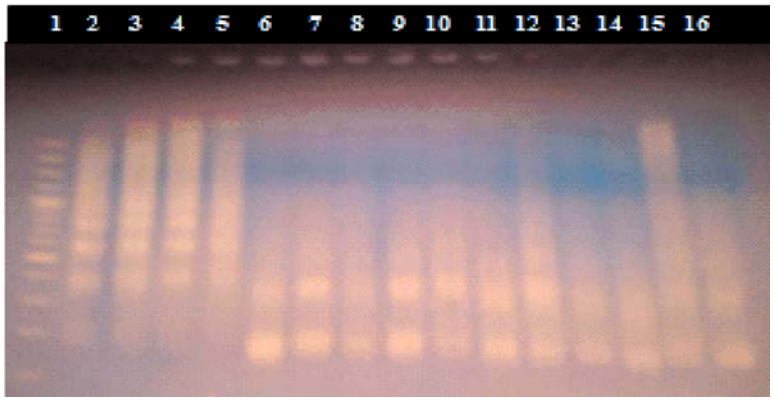

Fig. 2: Agarose gel electrophoresis of DNA samples extracted from liver tissues of different groups of adult rats.

Histopathological Examinations

Testes

Atrazine Group: The microscopical examination revealed that seminiferous tubules appeared relatively small in size, irregular in shape and widely separated from each other (Fig. 1A). The germinal epitheliums of some seminiferous tubules showed vacuolar degeneration and necrobiotic changes resulted in exfoliation of necrotic cells within the lumen of tubules. Some tubules contained homogenous hyalinized eosinophilic materials with no sperm within the lumen (Fig. 1A). Moreover, multinucleated giant cells and cellular debris were seen within some tubules (Fig. 1 B). There was prominent focal interstitial oedema together with congestion of blood vessels (Fig.1C). The basement membrane of some tubules was greatly thickened. Leydig cells were fewer and showed necrobiotic changes. In addition to the above mentioned lesions, there was inhibition and arrest of spermatogenesis.

Selenium Group: The microscopical examination revealed that the histological picture of the testis was more or less similar to that of control group. The tubules were closely compacted to each other and regular in outline. Most of seminiferous tubules showed within normal spermatogenesis (Fig.1 D). Mitotic division was obvious among the spermatogonial cells in some tubules (Fig. 1 E).

Atrazine and Selenium Group: The seminiferous tubules showed within normal spermatogenesis. However, some of other tubules revealed advanced necrobiotic changes of spermatogonial cells and complete depletion of spermatogenic epithelium lining with exfoliation into the lumens (Fig.1 F). Leydig cells appeared within the normal limit. Mitotic division was seen in some tubules in addition to presence of few multinucleated giant cells within the lumen of some tubules.

Prostate Gland

Atrazine Group: Most of the prostatic acini showed marked hyperplasia of the epithelium lining which was projected into the lumens of acini (Fig.1 G) associated with thick smooth muscle fibers (myoepithelium). They were loosely arranged in the stroma. There was dilatation and congestion of the most of intertubular blood vessels. Flattening of the epithelial lining and cystic dilatation of some acini was observed (Fig.1 H). The secretions in the acini were pale and eosinophilic.

Selenium Group: Showed normal acini with normal folded epithelial layer. The epithelium of these acini is basophilic and mostly columnar with occasional cuboidal cells that have basally located nuclei (Fig.1 I). The acini are rather tightly packed together within the supporting stroma. Secretions found in the acini stain very intensely with eosin.

Atrazine and Selenium Group: Prostate of rats treated with atrazine and selenium revealed less prominent histopathological changes as compared with atrazine group (Fig. 1 J). There was mild hyperplasia of epithelial lining of some of the prostatic acini. Few acini with cystic dilatation were seen.

DNA Fragmentation Analysis: Lane (1) represents 100 bp DNA marker, lanes (2 - 5) represent DNA from liver of atrazine treated rats, lanes (6 – 10) represent DNA from liver tissue of selenium treated rats, lanes (11 – 15) represent DNA samples from liver tissues of atrazine with selenium treated rats, lane (16) represents DNA from liver of control rats.

The DNA extracted from liver tissue of atrazine treated rats (lanes 2 - 5), shows high degree of fragmentation represented by ladder like shape using

1.5% agarose gel electrophoresis. While the DNA of liver tissues from rats treated with selenium shows low degree of fragmentation (lanes 6 – 10). At the same time, the atrazine with selenium treated rats DNA (lanes 11 - 15) shows high smearing and fragmentation in comparing with control one.

DISCUSSION

In the current work, MDA level and SOD activity increased significantly in rats treated with atrazine when compared to control group. Moreover, co-administration of selenium induced a significant decrease in MDA level as compared with atrazine treated animals. These results are in agreement with Campos-Pereira *et al*. [8] who found a significant increase of MDA levels and consequent lipid peroxidation indicative of plasma membrane damage. In contrast [32] reported non-significant MDA changes in male rats received 200 mg/kg body weight atrazine by oral route for one month. The increase in lipid peroxidation following atrazine exposure may be attributed to the induction of ROS which enhances the oxidation of polyunsaturated fatty acids that lead to lipid peroxidation [33]. The significantly attenuated oxidative stress produced by selenium could be attributed to the fact that selenium as a powerful antioxidant can protect cells from oxidative stress [34] by expression of selenoprotein genes and anti-inflammatory mechanisms [35]. The increase in SOD activity after atrazine administration in the present study appears to be an adaptive response to increased generation of reactive oxygen species. SOD protects against oxidative stress by converting free radicals or reactive oxygen intermediates to non-radical products [36].

In the present study, exposure to atrazine in male rats was associated with a reduction in blood testosterone levels with non- significant increase in plasma estradiol concentration. The decrease of testosterone secretion could be the result of decreasing the activities of pituitary-testis axis by atrazine through reduction of LH and consequently testosterone secretion [37]. Atrazine directly binds to steroidogenic factor 1 (SF-l) and decreases testosterone secretion by inhibition of SF-l receptors [38]. Testis is one of the main target organs for selenium which plays an important role in the male reproductive system [39]. The results of current study revealed that administration of selenium with atrazine has elevated the reduced blood testosterone levels. Selenium is needed for normal testosterone biosynthesis of testosterone and metabolism as well astesticular morphology which may explain the presence of several other selenoproteins in the male gonads [39]. Present results were consistent with findings of Trentacoste *et al*. [40] and Friedmann [41] who reported that atrazine exposure reduced circulating levels of testosterone in male rats.

The blood levels of AST and ALT are used as markers of the cell membrane integrity [2]. Present study revealed that rats treated with atrazine had significantly increased aspartate amino transferase (AST) and alanine amino transferase (ALT) activities as compared with control group. In addition, administration of selenium with atrazine resulted in a significant reduction in levels of both AST and ALT. The significantly higher activities of liver enzymes AST and ALT in rats exposed to atrazine are probably resulting from hepatocyte membrane damage and leakage of aminotransferase enzymes from injured liver cells [42]. Adesiyan *et al*. [43] reported that selenium therapy effectively combats the toxic effects of atrazine in the liver and thereby, selenium administration could be beneficial in attenuating the hepatotoxicity of atrazine. The obtained results are in agreement with AL-Attab and AL-Diwan [42] and Hussain *et al*. [44]. On the contrary, another study reported that rats treated with 400 mg / kg atrazine for 14 consecutive days result in non-significant elevation in serum ALT enzyme [8].

Histopathological examination of the testes of rat treated with atrazine revealed necrobiotic changes and vacuolation of spermatogonial cells. In addition, Leydig cells were fewer in number with necrobiotic changes. Similar results were recorded by Feyzi-Dehkhargani *et al*. [45] in rats and Sharma, *et al*. [46] in goats who observed pyknotic and fragmented nuclei, chromatolysis, hyalinization and condensation in spermatids *in vitro* after atrazine exposure. Atrazine exerts its effects primarily via the hypothalamic- pituitary-testicular axis by decreasing the synthesis of FSH and LH and testosterone concentrations and in turn, this impairment leads to Leydig and Sertoli cells dysfunction respectively and decreases the spermatogenesis processes [37, 45]. The pathological changes in testes of atrazine treated rats in the present study associated with reduced testosterone concentration as well as increased lipid peroxidation indicating that atrazine acts as an endocrine disrupter in male rat by directly inhibiting Leydig cell testosterone production [43]. In addition, atrazine impairs reproductive function through induction of oxidative stress [7]. Selenium is a constituent of selenoproteins that protect against oxidative damage to spermatozoa throughout the process

of sperm maturation and serve as structural components of mature spermatozoa [39]. In the current study, the pathological picture of the testes of rat treated with selenium and atrazine showed most seminiferous tubules with normal spermatogenesis as well as spermatogonial cells including Sertoli cells. In this respect, [39] reported that spermatogenesis was restored by feeding the selenium-adequate diet. Testicular morphology and functions are affected by severe selenium deficiency which is necessary for the formation and normal development of spermatozoa and consequently testosterone secretion. Development of male reproductive tissue requires an optimal level of selenium in testis and a small deviation either deficiency or excess leads to abnormal development [47]. On the contrary [43] mentioned that selenium had no protective effect against atrazine induced alterations in testes and epididymis.

Concerning the effect of atrazine on the degree of DNA fragmentation, our results showed that atrazine alone severely damaged hepatic DNA. Selenium administration partially reduced the genotoxic effect of atrazine denoted by low smearing of DNA in comparison with DNA of control one. These findings confirmed our previous study of atrazine genotoxicity in rat [32]. Supporting evidence for the genotoxic effect of atrazine comes from observation of DNA strand breaks in rat stomach, liver and kidney but not in lungs, after oral administration of high toxic doses [48]. The DNA disintegrity in present experiment may be due to oxidative damage in liver cells [2] associated with increased lipid peroxidation or due to impaired chromatin packing resulted from atrazine genotoxicity [45]. Coincidence with our results, atrazine caused significant increase in frequencies of micronuclei and percentage of DNA strand breaks in fish [49] and resulted in significantly longer comet tails of DNA damage in isolated hepatocytes of Japanese quail [44]. In contrast with our findings, mutation studies following in vitro exposure of mammalian cell lines to atrazine were negative; gene mutation studies on atrazine metabolites were also negative [50]. In the same context, the testes with atrazine also evidenced a non gene intoxication for mammalian cells *in vivo* and in vitro, although some positive results for tests of chromosomal aberration and of DNA damage and formation of micronuclei in rat and mice [51]. Selenium has antioxidant capacity and protective effect against wide range of agents induced oxidative stress [52]. Therefore, it can protect to some extant against atrazine toxicity represented by decreased degree of DNA fragmentation when dosed with atrazine. In line, [43] suggested that selenium effectively attenuated the toxic effects of atrazine-induced liver changes in rats. [53] reported that selenium might be protective by preventing DNA damage from occurring as well as by increasing the activity of repair enzymes such as DNA glycosylases and DNA damage repair pathways.

It can be concluded that selenium offered protection against atrazine induced lipid peroxidation and adverse effects on liver enzymes and reproductive performance in male rats. Selenium co-administration had weak protection against atrazine induced DNA genotoxicity.

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