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Histopathological and ultrastructural effects of methyl parathion on rat testis and protection by selenium

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ABSTRACT

The present study was conducted to evaluate the possible protective effect of selenium in reversing methyl parathion -induced testicular damage in male rats, using light and electron microscopy with reference to plasma testosterone (T) and lutenizing hormone (LH) levels. The animals were randomly divided into four groups, Group 1: control animals. Group 2: animals were treated with sodium selenite in a dose (10µg/kg b.w). Group 3: rats were given methyl parathion in a dose (0.28 mg/kg b.w). Group 4: rats were received sodium selenite prior to treatment with methyl parathion. Results showed that animals exposed to methyl parathion displayed a reduction in body and testicular weight and a reduction in seminiferous tubules diameter. At the end of the treatments plasma testosterone and LH concentrations were reduced significantly in methyl parathion-treated rats. Light and electron microscopy of testes from treated animals with methyl parathion showed disorganization of tubular elements with increased intercellular space. Also, revealed necrosis and cellular damage. In addition, a significant increase in sperm DNA fragmentation was detected. Selenium supplementation to rats given methyl parathion improved the testicular damage. In conclusion, Selenium may be useful for the prevention and treatment of methyl parathion -induced testicular damage.

INTRODUCTION

Organophosphorus (OP) compounds represent the most widely used group of pesticides for the control of agricultural pests and disease vectors (Maitra and Mitra, 2008). Due to the wide availability of OP compounds, poisoning is common (Garcia *et al.,* 2003). People are commonly exposed to OP pesticides through eating fresh and processed vegetables, contacting pesticide-contaminated surfaces, breathing air near pesticide applications (both indoors and outdoors), and drinking pesticide-contaminated water. In addition to its hazardous effects, OP pesticides compounds are known to cause adverse effects in male reproduction system (Uzunhisarcikli *et al.,* 2007). Methyl parathion (C8H10NO5PS) is an OP insecticide with a broad range of activity which inhibits acetyl cholinesterase activity (WHO, 1993). It has been widely and effectively used throughout the world with applications in agriculture and horticulture for controlling insects and in crops, cotton, corn, cabbage, potatoes, wheat and soybean (EPA, 1999). Methyl parathion has been reported to cause DNA damage in rats (Bartoli, *et al.,* 1991).

A significant increase in the percentage of abnormal sperm was observed in mice treated orally with methyl parathion (Mathew *et al.,* 1992) and caused a significant decrease in sperm count of rats (Narayana *et al.,* 2005). Free radical generation is expected to induce testes atrophy. Therefore, supplementation of antioxidants can be considered as the alternative method for chelation therapy. In fact, several studies demonstrated that the cellular antioxidant activity is reinforced by the presence of dietary antioxidants (Kiefer *et al.,* 2004). Accordingly, interest has recently grown in the role of natural antioxidants used as a strategy to prevent oxidative damage as a factor in the path physiology of various health disorders (Koechlin-Ramonatxo, 2006, Mehmetc- ik *et al.,* 2008& Shireen *et al.,* 2008). Among antioxidants, selenium (Se), used as nutritional supplements, are the essential elements in almost all biological systems. Furthermore, Akhtar *et al.*,(2009) reported that Se has the ability to counteract free radicals and protect the structure and function of proteins, DNA and chromosomes against oxidation injury .Synergistic effect of antioxidants such as Se is most powerful in reducing storage and toxicity of reactive oxygen species (Aslam *et al.,* 2010). The essential trace element selenium is the important component of cellular antioxidant defense and is involved in the modulation of intracellular redox equilibrium with

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its some 25 forms of cellular selenoproteins, particularly with glutathione peroxidases (GPx), and thioredoxin reductases (TrxR) (Oberley *et al.,* 2000). Selenium is actively involved in many fundamental biological processes ranging from immune functions to apoptosis, and protection and repair of DNA (Ganther, 1999). It is essential for the production of normal spermatozoa and thus plays a critical role in testis, sperm, and reproduction (Flohé, 2008). Also, he added that, the major role of Se in fertility is mediated by the membrane bound phospholipid hydroperoxide glutathione peroxidase (GPx4) which is the most abundant selenoprotein in testis.

Testis Se is known to be remarkably and preferentially maintained in Se deficiency. Severe and prolonged deficiency results in sterility as spermatogenesis was arrested, whereas in less severe Se deprivation reduced sperm motility leading to impaired fertilization capacity and abnormal sperm morphology were reported (Maiorino *et al*.,2006). The function of selenium is mediated mostly by selenoproteins, into which selenium, as selenocysteine, is inserted during translation (Kryukov *et al.*, 2003). Selenoproteins include enzymes such as glutathione peroxidases (GPXs), thioredoxin reductases, deiodinases, selenophosphate - synthetase 2, selenoprotein H (Reeves and Hoffmann, 2009). In this study, we examined the effect of methyl parathion on body and testis weights, sex hormonal status, histopathology and fine structure and assessed the protective potential of selenium (Se) in the rat.

MATERIALS AND METHODS

Chemicals

Methyl parathion (MP), purity 98%, was obtained from Sigma Chemical Co. (St. Louis, MO, USA)**.** Sodium selenite was supplied by Merck, Germany. All other chemicals were of analytical grade and were purchased from standard commercial suppliers.

Animals and treatment

In this study, forty healthy male adult sexually mature, albino rats (*Rattus norvegicus*) weighing approximately 190 ± 6 g; were purchased from the animal breeding colony of the High Institute of Public Health, Alexandria University, Alexandria, Egypt. The animals were acclimatized for a week before the onset of experiments under standard laboratory conditions (Temperature was 25ºC and humidity was 50% with a12:12h light and dark cycle) and those conditions were preserved till the end of the experiment. All animals were housed in plastic cages; animal cages were kept clean, and were given a commercial standard rodent diet (20% casein, 15% corn oil, 55% corn starch, 5% salt mixture and 5% vitaminzed starch; Egyptian Company of Oils and Soap Kafr-Elzayat Egypt) and fresh drinking water *ad libitum* regularly every day. All experimental procedures were conducted in accordance with the guide lines of Animal Ethics Committee of the Alexandria University. The animals were randomly divided into four experimental groups of 10 rats each:

Group1: control animals which given corn oil.

Group2: animals were treated with sodium selenite. Sodium selenite was dissolved in water and was administered to rats by gavage in a dose (10µg/kg b.w) daily for 8 weeks (Swathy *et al.*, 2006).

Group3: rats of this group were given methyl parathion. Methyl parathion was administered to rats in a dose (0.28 mg/kg b.w) in corn oil through gavage once a day for 8 weeks (Uzunhisarcikli *et al.*, 2007).

Group4: rats of this group were received sodium selenite prior to treatment with methyl parathion daily for 8 weeks. Rats were sacrificed by cervical dislocation after the end of the treatment.

Measurement of body and organ weights

During the treatment period (8 weeks), food and water intake, body weight of the animals were monitored daily. Body and testis weights of control and treated rats were measured at the end of the experiment by automatic balance (AND GX-600, Japan).

Hormone assays

The blood samples were obtained from the animals by heart puncture. The serum level of testosterone was determined according to; Abraham (braham ,1977), luteinizing hormone was detected according to, Jaffe & Behrman (1974).

Histopathological examinations

Animals were cervical decapitation and the organs/tissues were carefully examined macroscopically and gross lesions were recorded. Testes from each animal were placed in Bouin's fixative and slides prepared for histopathological examination. Histological examination was conducted by using routine paraffin embedding technique. Sections of 4 µm thick were performed in a microtome Leitz 1512 (Leitz, Wetzlar, Germany) and stained with hematoxylin and eosin (Luna, 1968) were examined under a light microscope (Olympus, Tokyo, Japan). To perform the morphometric analysis of a total 50 tubules per group, mean diameter of the seminiferous tubules and the height of the germinal epithelia were determined using an ocular micrometer. To evaluate spermatogenesis, twenty of the roundest tubules per testicular section were selected and calculated. Johnsen's mean testicular biopsy score (MTBS) was used to evaluate the histopathologic changes in 20 seminiferous tubules of each testicular section for these observations, an objective lens (magnification: 40x) was used. The Johnsen's method (Johnson *et al.*, 1980) applies a score of 1–10 for each tubule cross-section, whereby a score of 10 indicates complete spermatogenesis and perfect tubules, while a score of 1 means no germ cells and no Sertoli cells present. The Johnsen's score per tubule was expressed as mean ± SEM for each group.

Electron microscopic analysis

Testes tissues were cut into small $1mm^3$ pieces, and immediately fixed in 2.5% glutaraldehyde in 0.1M phosphate

buffer, pH 7.4, post fixed in 1% OsO_4 in the same buffer, dehydrated in graded acetone solutions, and embedded in epon. Semithin sections were cut at 1µm in thickness with a glass knife. They were stained with 1% toluidine blue dissolved in 1% borax for approximately 30s at 40–50 ºC and examined under the light microscope for general orientation. Ultrathin sections, 50–60nm in thickness were obtained from the selected areas using a Reichert OM U3 (Leica Co., Austria) ultramicrotome and the diamond knife. The grids were then stained with 2% uranyl acetate and lead citrate (Reynolds, 1963).

The sections were viewed and photographed on a Jeol 100 CX II transmission electron microscope (TEM) (Joel Ltd., Japan) at 60 - 80 kV. In Electron Microscope Unite, Faculty of science, University of Alexandria, Egypt. Electron micrographs were taken from selected areas.

DNA fragmentation

TUNEL assay

To confirm the genotoxic effect of methyl parathion, the presence of DNA strand breaks in spermatozoa was evaluated by terminal deoxynucleotidy l transferase (TdT) -mediated d UTP nick end labeling (TUNEL), using an in *situ* Cell Death Detection Kit, Fluorescein with slight modifications to the manufacturer's protocol.

Sperm cells were collected and were transferred to conical tubes to allow the sedimentation of larger pieces. Samples were then filtered through a200 mm nylon mesh to microtubes containing glycerol to a final concentration of 10%(v/v) and kept in an ultra-cold freezer (-80 ºC) until FCM analysis. Samples were thawed in crushed ice, centrifuged (500g for 6min)and washed twice in phosphate buffered saline (PBS)/1% bovine albumin serum (BSA)pH7.4.

One hundred mL of 1% freshly prepared paraformaldeheyde was added to an aliquot of 100 mL of sample and cells were fixed in a rotative shaker for 1hat room temperature. After that, cells were washed twice in PBS with1%BSA to remove the fixative and the newer permeabilized with 100 mL 0.1%TritonX-100 in 0.1% sodium citrate for 2 min on ice. After washing once with PBS with 1% BSA, the supernatant was removed and the labeling reaction was performed by incubation with 50 mL of labeling solution(supplied with the In Situ Cell Death Detection Kit) containing TdT for 1h at 37 °C in the dark. For each sample, a negative control was prepared by omitting TdT from the reaction mixture. After labeling, two subsequent washes in PBS/1%BSA were performed and the sample was re suspended in the same buffer to a volume of 500–700 mL, counters tained with PI to a final concentration of 0.5 mg/ mL, and measured by flow cytometry.

At least 10,000events were analyzed for each sample at a flow rate less than 200 cells /s, after gating the debris component in the S vs. FL3 characteristics of sperm cells. The following controls were used: as positive control, fixed and permeabilized sperm cells were incubated with 5 UDNAseI for 45 min at 37 °C before the labeling reaction, in order to induce DNA fragmentation; as negative control, fixed and sperm cells were prepared as above but were incubated with labeling solution without the TdT enzyme.

Statistical analysis

Data were analyzed using SPSS11.0 for Windows. Statistical significance was calculated using a one-way analysis of variance (ANOVA) followed by Tukey's procedure for multiple comparisons. $P < 0.05$ was considered statistically significant.

RESULTS

The effects of methyl parathion on general rat's health

All rats were clinically healthy before experiment. Death was not observed in any experimental groups during the treatment period (8 weeks). However, in methyl parathion treated group, few clinical signs such as reduced activity, increasing weakness, slight diarrhea and hair loss were observed.

Food intake

Food intake and water consumption of the methyl parathion -treated rats was reduced by 25% and 23 %, respectively. In the (methyl parathion + selenium)-treated group, the food intake increased significantly ($p<0.01$), when compared to methyl parathion group. No significant changes were observed in Se - treated group (Table 1).

Evaluation of body and organ weights

Body weight, absolute testis weight, and relative testis weight did not significantly differ during the experiment between the selenium - treated group and the control group. Body weight absolute testis weight and relative testis weight had significantly decreased in the methyl parathion treated groups, compared to the control group ($P<0.05$).

Administration of selenium to methyl parathion-treated rats preserved body weight and testicular weight, significant increased ($P <0.01$) were observed, when compared to methyl parathion group, by the end of the experiment (Table 1).

Changes of serum hormones (Testosterone, LH)

Testosterone and luteinizing levels in the control group were compared to those in the experimental groups at the end of 8^{th} weeks in the experimental groups: selenium, MP, and selenium +MP (tables 1&2).

In addition, the MP-treated group was compared to the selenium + MP-treated group. Changes in hormones (Te & LH) levels are shown in table 2. No statistically significant changes were observed between the selenium-treated group and the control group at the end of 8^{th} weeks. A significant decrease in Te & LH levels in the MP -treated group compared to the control group ($P < 0.05$) was observed. On the other hand, a significant recovery in testosterone and LH levels was observed in the selenium +MP-treated group compared to the MP-treated group at the end of the 8^{th} weeks.

Table 1: Changes in daily food and water consumption, Body weight, testis weight, and relative testis weight in different groups under study.

Parameters and treatments	Control	Methyl parathion	selenium + methyl parathion	selenium
Food intake (g/day/rat)	32.43 ± 3.36[a]	24.46 ± 3.09[b]	30 ± 4.48[a]	33.66 ± 2.61[a]
Water intake (mL/day/rat)	27.9 ± 2.90[a]	21.06 ± 3.1[b]	26.36±4.38[a]	28.03 ± 4.68[a]
Initial body weights (g)	193.63 ± 7.65	192.46 ± 5.81	190.16 ± 5.66	195 ± 6.06
Final body weights (g)	210 ± 4.93[a]	173 ± 4.79[b]	204.5 ± 7.79[a]	212.43 ± 5.04[a]
Absolute testis weights (g)	3.04 ± 0.02[a]	2.4 ± 0.59[b]	2.89 ± 0.08[a]	3.08 ± 0.03[a]
Relative testis weights (mg/g bw)	0.8733 ± 0.005[a]	0.7967 ± 0.01[b]	0.77 ± 0.012[a]	0.875+ 0.005[a]

Values are mean ± SE for eight rats in each group.
Values not sharing common superscript letters (a-b) differ significantly at $p<0.05$.

Table. 2: Effect of methyl parathion and selenium on the testosterone and luteinizing hormone concentrations in the testis in different groups under study.

Group (each = 8 rats)	Testosterone (ng/ml)	Luteinizing hormone LH (IU/L)
control	4.2 ± 0.41	1.8 ± 0.19
methyl parathion	1.9 ± 0.54[b]	0.6 ± 0.05[b]
selenium	4.1 ± 0.39	1.5 ± 0.18
methyl parathion +selenium	3.7 ± 0.37	1.2± 0.20

Values are mean ± SE for eight rats in each group.
b differ significantly at $p<0.05$ compared to control.

Table. 3: Effect of methyl parathion and selenium on spermatogenesis assessed by Johnsen's scoring system.

Groups	Johnsen's Score
Control	9.50 ± 0.54[a]
Sodium selenite	9.10 ± 0.6[a]
Methyl parathion	5.000 ± 0.817[b]
Sodium selenite + Methyl parathion	8.900 ± 0.817[a]

Values are expressed as mean ± SD. The difference between means that have different superscripted letters (a and b) is statistically significant at $P < 0.01$.

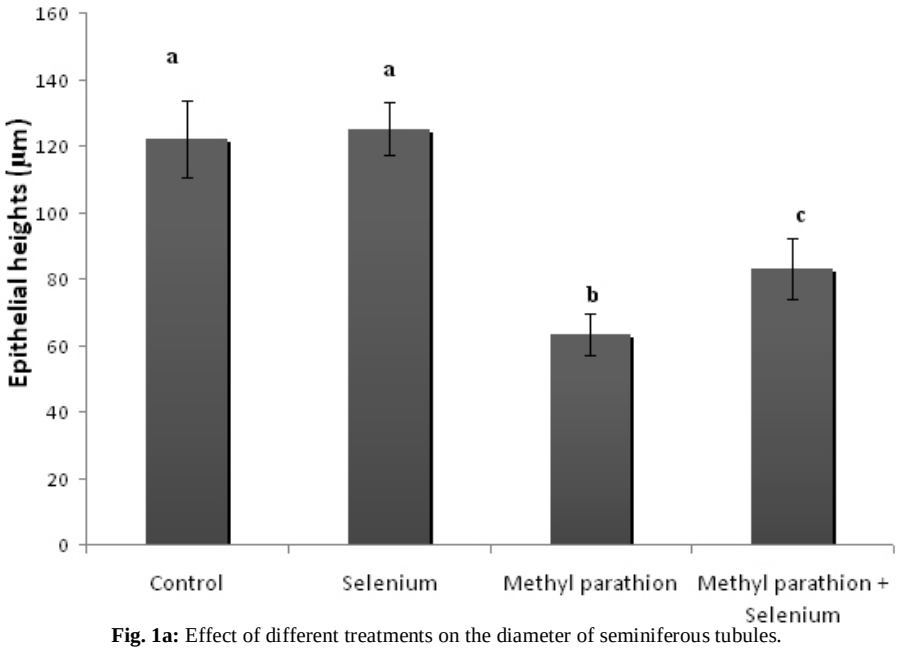

Fig. 1a: Effect of different treatments on the diameter of seminiferous tubules.

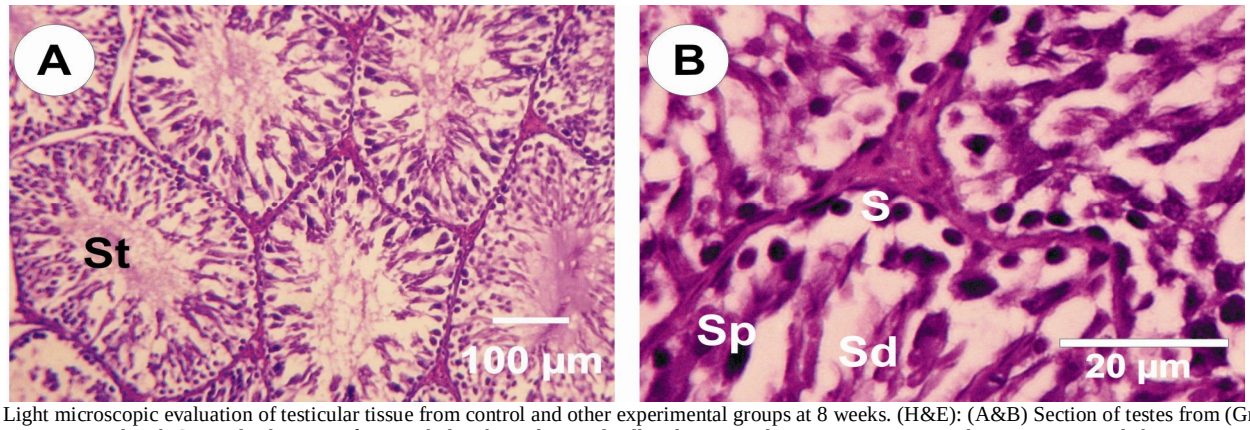

Fig. 2: Light microscopic evaluation of testicular tissue from control and other experimental groups at 8 weeks. (H&E): (A&B) Section of testes from (Group 1) rat treated with Corn oil. The seminiferous tubules showed normal cell and interstitial tissue associations without any structural changes.

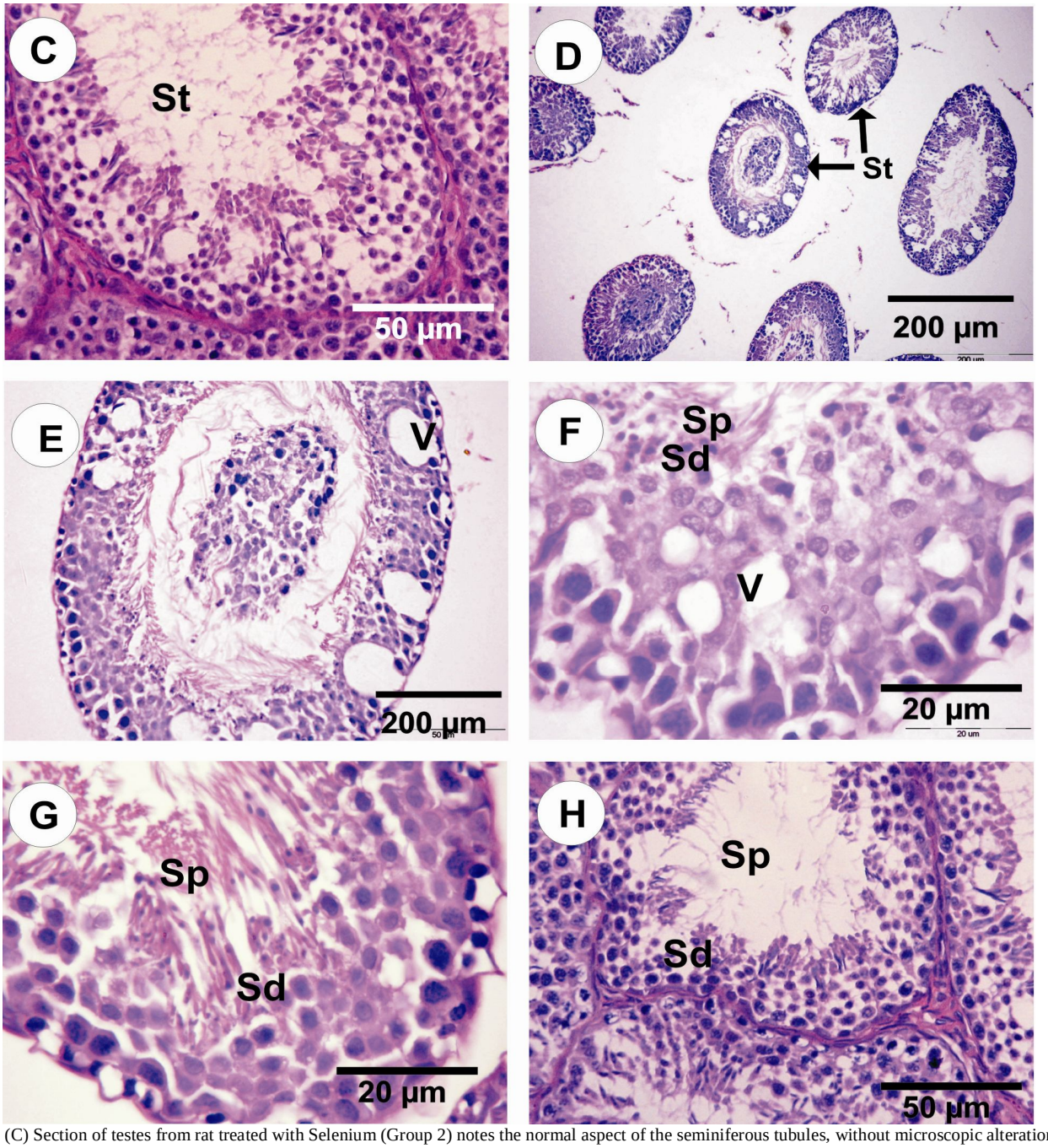

Fig. 2: (C) Section of testes from rat treated with Selenium (Group 2) notes the normal aspect of the seminiferous tubules, without microscopic alterations in structure. (D-G) Section of testes from (Group 2) rat treated with methyl parathion, (D) All seminiferous tubules showed either degeneration or atrophy; Interstitial tissues showed edema, hemorrhage and vacuolation. (E) Many seminiferous tubules with intact germinal layer and undergoing degeneration along with loss of spermatogenesis. (F&G) Note: that degenerating germ cells show nuclear pyknosis, wide gaps between neighboring cells with enlargement of the Intercellular spaces, restoration of spermatogenesis in most of the seminiferous tubules, necrotic germ cell sloughed out into the lumen of seminiferous tubules were seen. (H) No pathological changes in seminiferous tubules and interstitial tissue. Section of testes from (Group 4) rat treated with Methyl parathion plus Selenium. [Seminiferous tubules (st), Sertoli cell (S), spermatids (Sd), spermatozoa (SP) and vacuoles (V)].

Morphometric results

Figure (1A&B) showed the morphometric changes in the diameter of the seminiferous tubules and their epithelial heights. Treating rats with selenium for 8 weeks caused insignificant increase in the diameters of the seminiferous tubules compared with control group. Rats daily treated with methyl parathion for 8 weeks showed significant decrease ($P < 0.05$) in tubules diameters and their epithelial heights. On the other hand, animals treated with methyl parathion and selenium for 8 weeks showed highly significant increase ($P < 0.001$) in the diameter of seminiferous tubules and their epithelial heights in comparison with methyl parathion group.

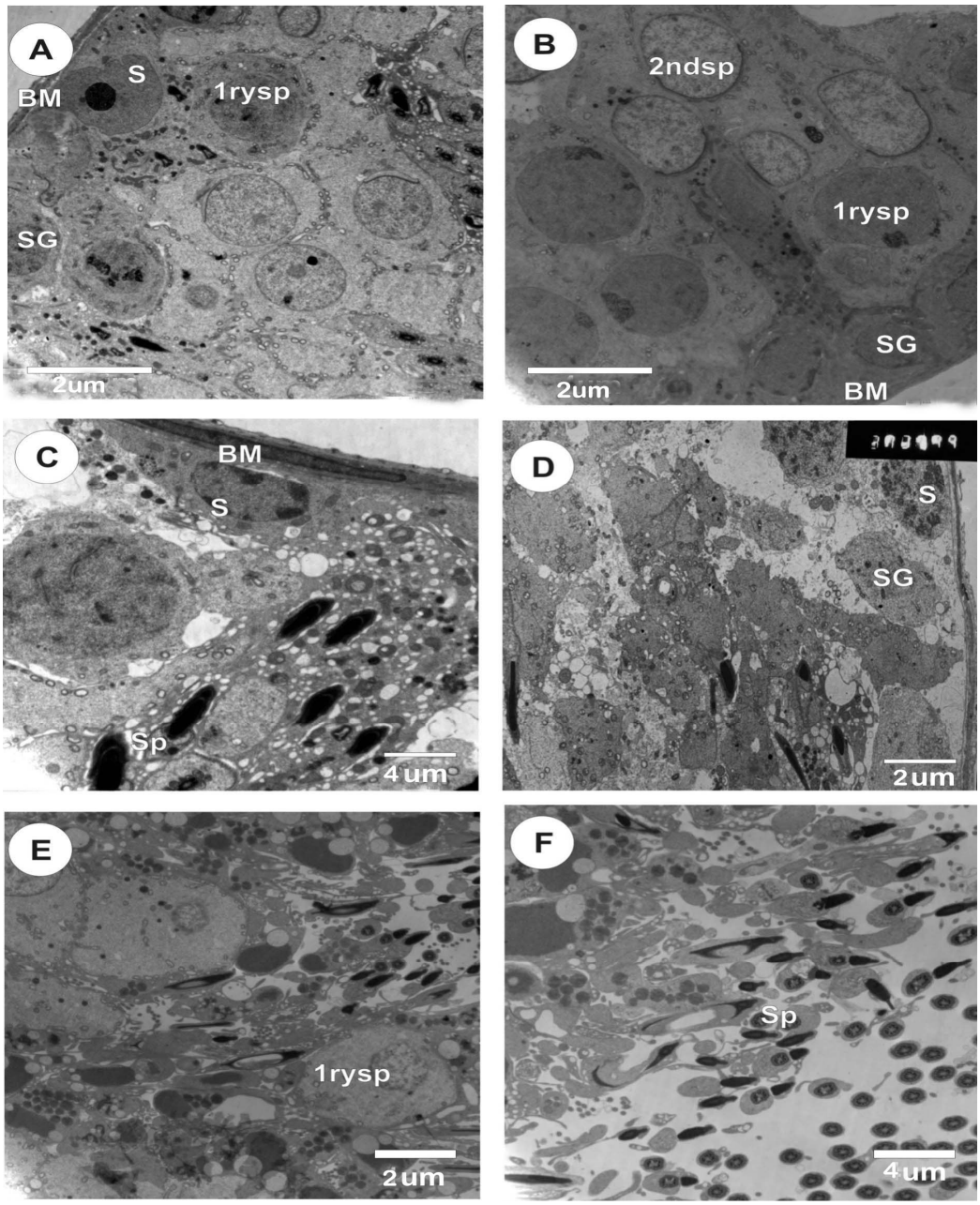

Fig. 3: Electron micrographs of control and MP treated testicular tissue of rats. A&B: seminiferous tubule of control and selenium rat .Note multilayered basal lamina, Sertoli cells with well-preserved organelles, tight cell to cell contact. B: spermatids in control rats with dense chromatin, normal acrosomal vesicles with prominent nuclei. C: testis of a rat treated with MP showing excessive damage to seminiferous tubules, disrupted basal laminae, enlarged intercellular spaces, vacuolated and dilated and abnormal Sertoli cell cytoplasmic organelles, spermatocytes with vacuolated cytoplasmic organelles, abnormal spermatocytes, pachytene spermatocytes, and absence of intercellular bridges. The cytoplasm contains abnormal vesicles (arrow) and has some marginal chromatins along the nuclear membrane. Sertoli cell disruption and vacuolization regressed basal lamina. D: shows reduced spermatid nuclei with disrupted and irregular nuclear membrane. E, seminiferous tubule of (Group 4) rat treated with Methyl parathion plus Selenium showed normal testicular architecture with the regular course of spermatogenesis and mature spermatid. [Basement membrane (BM), Sertoli cell (S), spermatid (Sp)].

Histopathological changes

Figure (2A-H) illustrates the different histopathological changes in the testes of animals that were given various treatments. The testicular structure in the control (G-1) and Selenium (G-2) treated group at the end of the experiment was normal. The seminiferous tubules appeared uniform in size and shape. They were lined by regularly arranged rows of spermatogenic cells in different stages of maturation. Spermatogenic cells and Sertoli cells in the seminiferous tubules were observed in normal structure (Fig.2A, B&C, respectively). In methyl parathion-treated rats, the tubules were relatively wide and separated from each other with degenerated intertubular tissue (Fig. 2D). Necrosis, Vacuolization and disruption of the seminiferous epithelium especially at the basal compartment indicating their location in the Sertoli cells and edema in interstitial tissue were observed (Fig. 2E). Disorganization of the

seminiferous epithelium hypospermatogenesis (loss of germ cells from the seminiferous epithelium) could be seen. The germ cells showed nuclear pyknosis preceding degeneration and due to germ cell loss (Figs. 2F&G). Selenium was able to recover the structural effects induced by methyl parathion (group 4) to the control level. No histopathological changes were detected in seminiferous tubules and interstitial tissue in methyl parathion and selenium treated group at the end of the experiment (Fig. 2H). Johnsen's testicular biopsy score count, the scores were significantly decreased in the methyl parathion treatment group ($P < 0.01$) in contrast to that of the control group, indicating that spermatogenesis had been suppressed by methyl parathion. On the other hand, There was no statistically significant difference between sodium selenite, methyl parathion plus sodium selenite and the control group, when compared with each other in means of spermatogenesis. The count was significantly increased in the sodium selenite -included treatment group compared to the methyl parathion treatment group, suggesting that the methyl parathion - suppressed spermatogenesis was protected by sodium selenite (Table 3).

Electron microscopic observations

Ultrastructural observation of the control rats (Group I) and selenium-treated (Group 2) showed normal testicular architecture with the regular course of spermatogenesis and Sertoli cells. Seminiferous tubules were bounded by a normal basement membrane, thick and complete basement membrane. The spermatogonia were recognized as diploid cells situated at the boundary tissue of the seminiferous tubules, all stages of transformation of the seminiferous epithelium from spermatogonia to mature spermatozoa could be seen in the tubules (Figs. 3A&B). These were characterized by large ovoid nuclei containing finely granular nucleoplasm. The nuclei were present with their long axis parallel to the boundary tissue, the chromatin was homogenous and the cytoplasm was granular. The primary spermatocytes had spherical nuclei with finely granular nucleoplasm and clumps of chromatin. Normal morphology of Sertoli- cells were recognized by the cytoplasm that extended from the basal lamina to the lumen of the seminiferous tubules and enveloped the adjacent germinal elements. There were no degenerative changes or vacuolizations. At the ultrastructural level in MP treated (group3), showed severe necrosis and degeneration of seminiferous tubules, all atrophic tubules showed changes in the basal lamina, including irregularity and indentation (Fig. 3C). Also, testes showed partial loss of the spermatogenic cells. Intensified sloughing of immature cells was frequently seen. The germ cell arrangement within the seminiferous tubules was disrupted. The Sertoli cell nuclei were dislocated from the basal portion with vacuoles, dense material and whorled structures in their nucleoplasm (Fig. 3D). There was extensive coalescence of vacuoles in and between Sertoli cells and spermatocytes with signs of germ cell depletion and exfoliation (Fig. 3E). Numerous vesicles and electron dense materials in their germ cell cytoplasm. Some marginated chromatins along the nuclear membrane and mitochondrial distension were frequently recognized within the germ cell cytoplasm of MP treated rats. Damaged elongated spermatids could be seen most probably surrounded by germ cells were widely separated and only few spermatocytes and cap stage spermatids could be recognized (Fig. 3F). The population of the detected developing spermatids showed deleterious abnormalities. (Fig. 3G). Ultrastructurally, selenium treatment in MP administered group showed significant improvement in their germ cell morphology (Fig. 3H).

DNA fragmentation

A tendency to an increase was detected in the percentage of sperm cells with fragmented DNA, assessed by TUNEL assay, in methyl parathion-treated rats, the differences reached statistical significance ($p < 0.05$) (Fig.4). Administration of selenium exerted a significant protective effect against methyl parathion-induced DNA damage as evidenced by Figur.4. No appearance difference could be observed in DNA among the control group, the selenium group and the methyl parathion + selenium group.

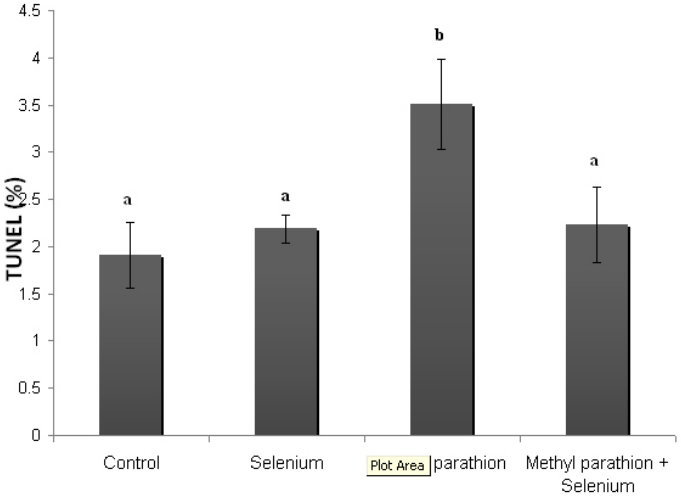

Fig 4: Effects of methyl parathion and selenium on sperm cell's DNA fragmentation assessed by the TUNEL assay.

DISCUSSION

Experimental and human epidemiological data indicated that the OP compounds, including methyl parathion, being lipid soluble, can be quickly absorbed from the skin, gastrointestinal tract, and especially pulmonary route. As these chemicals can easily pass through not only the blood-brain barrier but also the placental barrier, they can cause disorders of the fetal organs when they enter the maternal reproductive system during pregnancy (Desi and Nagymajtenyi, 1999). Mammals are expected to be adversely affected by methyl parathion through oral, dermal and inhalation exposure pathways (Garcia *et al.*, 2003).

In the present study, even though MP was given at 1/50 LD50 dose orally, we observed pathological findings in rat testes. But no rats died during the experimental period. Many studies imply that OP insecticides cause reduction of body and organ weights in experimental animals (Ogutcu *et al.*, 2006 and Kalender *et al.*, 2007). In the present study, reduced body and testis weights

were observed in animals given MP. This may be attributed to the decrease in food intake, as recorded in the present work. In addition to this, significant decline in testicular weight may be due to decrease in the number of spermatogenic cells (Joshi *et al.*, 2004).

Furthermore, in the current study, suppression of luteinizing hormone (LH) and testosterone concentrations and severe degeneration of spermatogenesis in testes were observed in MP-treated rats in comparison with control group. A decrease in testosterone concentration with MP treatment appears likely to be due to the down regulation of LH secretion implicating active suppression of testosterone secretion by the testes. Alternatively, as indicated by the histomorphological damage, seminiferous tubules were relatively wide and separated from each other with degeneration of peritubular tissue. Treatment with methyl parathion and selenium for 8 weeks caused significant increase in serum testosterone and LH in comparison with rats treated with MP. There was insignificant increase in levels of these hormones in sera of animals treated with selenium for 8weeks in comparison with control group. The secretion of testosterone in the Leydig cells is regulated by LH released from the pituitary gland. It is suggested that selenium had a stimulating effect on LH or on the biosynthesis of testosterone (Behne *et al.,* 1996). In fact, Se accessed the central nervous system via cerebral capillary seleno-protein receptors (Wilson *et al.,* 2002) and could be incorporated in newly synthesized selenoproteins. According to Santos and Takahashi (Santos and Takahashi, 2008). Se supplementation increased the activities of selenoproteins, by the high incorporation of selenocysteine in selenoproteins which may decrease free radical-mediated LPO and regenerate glutathione.

Pesticides cause various histopathological and cytopathological changes in the male reproductive system (Sarabia *et al.,* 2009). In the present study, rats exposed to methyl parathion pesticides displayed pronounced histological changes in their testes. The seminiferous tubules showed various degrees of pathological lesions and degenerative changes. Microscopic examination of sections of the testes from methyl parathion-treated group showed collapsed and shrinkage seminiferous tubules. In addition, the diameter of seminiferous tubules was decreased in size. These results similarly observed by Debnath and Mandal (2000) who reported, that organochlorine chlordane exposure in rats produced damage in the testicular tissues and reduction in the diameter of the seminiferous tubules.

Also, the present study revealed disarrangement of the regular succession of germ cells in rats from the methyl parathion group. Furthermore, germ cells were sloughed off from their normal position. Similarly, benomyl fungicide caused sloughing of germ cells in mice (Hess and Nakai, 2000). In the present study, after methyl parathion exposure, it caused apparent necrosis and edema in the seminiferous tubules and interstitial tissue and degeneration of spermatogenic cells with apoptosis and necrosis. Methyl parathion may prevent spermatogenesis through passing blood-testes barrier and it may decrease sperm production (Poet *et al.,* 2004). Ultrastructural examination of testes of MP-treated animals showed marked damage in the cytoplasmic organelles in most cells of the seminiferous tubules. Published data showed degenerative changes of spermatogonial cell and spermatocytes following dimethoate and volaton treatment in the rats (Atef *et al*., 1995). In parallel, Kniewald *et al.,* (2000) reported disintegration and necrosis of spermatogenic elements in testes of mice under the influence of atrazine. Mitochondria are the key organelles representing cellular damage (Kalender *et al.,* 2005) and pesticide derived mitochondrial pathologies are well known (Joshi *et al.,* 2003). Abnormaly enlarged acrosomal vesicles, abnormal chromatin condensation; multiple vacuoles in the nucleus and degenerated cytoplasm were among spermatid abnormalities found in methyl parathion group. In some microscopical preparations, accumulation of immature and necrotic spermatids was frequently seen in the lumen of the seminiferous tubules. This also has been reported by Sakr and Azab (2001) who observed a significant reduction of intraluminal sperm concentrations following pyrethroid treatment.

Sertoli cells rest on the seminiferous epithelial basal lamina and reach the lumen. They undergo histopathological and biochemical changes by the action of toxicants. Pesticides are a major toxic class of chemicals which are known to target Sertoli cells. It is believed that the Sertoli cells are mediators for all metabolic exchange with the systemic compartment (Martinage *et al.,* 1996). The present ultrastructural study revealed a marked damage of the Sertoli cells, from the methyl parathion group, thus suggesting alteration of their function. In some specimens, Sertoli cells were hypertrophied. In other specimens, ultrastructural changes of Sertoli cells included vacuolization and disintegration of cytoplasm, increase in number and size of cellular debris of damaged germ cells, and increase amount of lipoid droplets. Disruption of the tight contacts between Sertoli cells and neighbouring germ cells was obviously revealed in specimens of the methyl parathion group.

Several techniques have been used to evaluate DNA strand breaks in spermatogenic cells, among them, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) (Ahmadi and Ng 1999). In the present work, the effects of methyl parathion on rat spermatozoa DNA fragmentation were evaluated. Sperm cells from rats exposed to methyl parathion showed higher levels of DNA fragmentation detected by TUNEL assay. Similarly, Sánchez-Peña *et al*.,(2004) Observed alterations on sperm chromatin structure and DNA integrity in agricultural workers exposed to OP. Recio *et al.,* (2001) reported chromosomal damage expressed as increased frequency of aneuploidy after occupational exposure to a mixture of OP, including Me–Pa.

Oxidative stress is induced by oxidant substances commonly known as ROS (Sheweita *et al.,* 2005). Various data have shown that ROS were involved in the modulation of cell redox state, and redox regulation of protein functions is now accepted as an additional regulatory mechanism of normal cell physiology (Veal *et al.,* 2007). Thus, ROS recently gained attention as important second messengers. However, excessive production of ROS may lead to oxidative stress, loss of cell

function, and cell death by apoptosis or necrosis (Nose, 2000). Also, the induction of oxidative stress was previously suggested to represent a common mechanism in endocrine disruptor-mediated dysfunction, specific to certain testicular cells (Latchoumycandane and Mathur, 2002). Excess levels of reactive oxygen and nitrogen species can attack biological molecules such as DNA, protein and phospholipids which led to increase of lipid peroxidation and depletion of the antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) (Sies,1985). MP induced teaticular toxicity in rats; this may be due to its oxidative stress. In this concern, Abu El-Saad (2011) reported that examination of testes homogenates of rats treated with methyl parathion indicated a significant increase in malondialdehyde and reduction in the activities of the antioxidant enzymes (SOD and CAT) which lead to impair the antioxidant defense and increased lipid peroxidation levels in the testis. A change in the cellular antioxidant status and MDA level for that study was regarded as an indicator of increased ROS production, which is attributed to the oxidative damage in cellular macromolecules such as lipids, proteins and nucleic acid of testicular tissues. Selenium supplementation resulted in improvement of histological as well as biochemical alterations induced by MP. Dietary antioxidants supplementation has been proved to have protective effects against oxidative damage by reinforcing cellular antioxidant defenses (Ben Amara *et al.,* 2011). Organic forms of selenium have been suggested as possible antioxidant agents (Nogueira *et al.,* 2004). At low concentrations, Se compounds are antigenotoxic and anticarcinogenic, whereas at high concentrations, they are mutagenic, toxic, and possibly carcinogenic (Letavayová *et al.,* 2006). In our case, the administration of selenium to the methyl parathion-treated group improved body and testis weights, which could be attributed to an increase in daily food consumption. Indeed, Navarro-Alarcon and Cabrera-Vique (2008) and Ismail *et al.*(2009) showed an activated growth and development after supra- nutritional intake either of selenium or vitamin E.

Selenium plays a critical role in testis, sperm, and reproduction. In rodent testis, Se concentrations are typically higher than for any other tissue except kidney, and unlike most other tissues except brain and endocrine tissues, generally do not decrease even with prolonged Se deficiency, showing that Se is preferentially maintained in the rodent testis (Behne *et al.,* 1988). Selenoprotein P (Sepp1) is the responsible molecule for the targeted trafficking of Se to testis (Hill *et al.,* 2003 and Schomburg *et al.,* 2003). When selenium supply is limited due to selenium deficiency, incoming selenium as Sepp1 would be delivered preferentially to the Sertoli cells, thereby maintaining testis Se concentrations. Selenoproteins identified in testis are Sepp1, mitochondrial and cytosolic TrxR, GPx1, and GPx4 as themost abundantly present selenoprotein in rat testis (Maiorino *et al.,* 2006). It is thought that the seminiferous epithelium and mature sperm also require a particularly efficient protection against oxidative stress (Zini and Schlegel, 1997). GPx1, as the selenoperoxidase most efficient in H2O2 reduction would indeed be the enzyme of choice to meet this demand (Ursini *et al.,* 1995). Sakr *et al.,* (2011) reported that sodium selenite supplementation significantly protected against carbimazole induced testicular gametogenic and spermatogenic disorders, prevented testicular oxidative stress and increased antioxidant status.

Jana *et al.,* (2008) reported that sodium selenite supplementation significantly protected against exercise induced testicular gametogenic and spermatogenic disorders, prevented testicular oxidative stress and increased antioxidant status. Grotto *et al.,* (2009) examined the possible antigenotoxic effect of selenium in rats chronically exposed to low levels of methyl mercury. Selenium co-administration showed a significant reduction in methyl mercury-induced genotoxicity, re established glutathione peroxidase activity and reduced DNA damage. Kara *et al.,* (2007) concluded that selenium is effective in reducing the genetic damage induced by the anti tumor agent ‘doxorubicin’ in vitro in human lymphocyte. The authors added that the mechanism of chemoprotection of selenium may be related to its antioxidant properties as well as its ability to interfere with DNA repair pathways. Guttenplan *et al.,* (2007) showed that the organo selenium compound, 1, 4-phenylenebis (methylene) selenocyanate inhibited 4-nitroquinoline-N-oxide (4- NQO)-induced tongue tumorigenesis and DNA damage in the tongue of Fisher rats. Also, Guttenplan *et al.,* (2007) reported that selenium diminished DNA damage induced by incubation of calf thymus DNA with diazinon. In conclusion, methyl parathion, showed reproductive toxicity in male rats and selenium has a protective role on this toxicity. This may be attributed to the antioxidant activity of selenium.

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