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Malathion-induced testicular toxicity is associated with spermatogenic apoptosis and alterations in testicular enzymes and hormone levels in male Wistar rats

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Highlights

- Assessment of the reproductive toxicities in male rats exposed to malathion for 60 days
- Malathion induced degenerative changes in seminiferous testicular tubules
- Apoptosis rate increased in spermatogenic cells in malathion-treated rats
- Increased expression of Bax and decreased expression of Bcl-2 were observed in rats
- Malathion induced abnormal activities of testicular enzymes and reproductive hormones

ABSTRACT

Malathion has a broad range of toxicities while its reproductive effects have not been fully elucidated. In this study, we treated animals with malathion by gavage at doses of 0, 33.75, 54, and 108 mg/kg for 60 days and evaluated the alterations in histology, biochemistry and serology. Malathion caused the reduction in the sperm counts and motility. The reduced body and testis weights were coupled with mild to severe degenerative changes in seminiferous tubules. We

found malathion at 54 mg/kg increased spermatogenic apoptosis rate which was confirmed by changes in protein expression of Bax and Bcl-2. The activities of testicular enzymes including ACP, LDH and γ-GT were significantly altered with the reduced level of reproductive hormones such LH, FSH and T. These results indicate that malathion can elicit deleterious effects on reproductive system of rats. The abnormal levels of hormones and apoptotic proteins induced by malthion may play important roles.

Key words: Malathion; reproductive effects; histopathological changes; testicular enzymes; apoptosis; reproductive hormones

1. Introduction

Malathion is an organophosphorus (OP) pesticide widely used for controlling pests in live stock and agricultural and garden plants. Residents near the farms and farmworkers may be exposed to organophosphate pesticides due to their common use in agriculture and households [Quirós-Alcalá et al 2011; Ojha and Srivastava, 2014]. The main exposure routes include

ingestions through contaminated food and drinking water [Flores-Garcia et al, 2011; Sapbamrer and Hongsibsong, 2014], inhalation during production, handling and application of insecticides [Machera et al, 2003], and dermal contact with contaminated soil and plants and from accidental spills [Tuomainen et al, 2002]. Acute exposure to OP insecticides and subsequent OP poisoning with muscle dysfunction can be a cause of deaths in humans due to mitochondrial dysfunction [Karami-Mohajeri et al, 2014]. Long term exposure to OP pesticides has been proved to impose higher risks of various chronic diseases [Mostafalou and Abdollahi, 2013].

Malathion is widely used because of its relatively low acute toxicity compared to other OP insecticides. Extensive studies have been conducted for assessing the potential health effects of malathion in a variety of biological models from amphibians to mammalians. It has been reported that malathion induces toxicity through the inhibition of acetylcholinesterase (AchE) and subsequent activation of cholinergic receptors [Lasram et al, 2008]. Animal study suggested that inhibition of enzymes leads to age-dependent neurologically disorders neurological and behaviour disorders [Vidair, 2004]. Moreover, many OP pesticides including malathion have been identified as endocrine disruptors (ED) which can interfere with hormone levels through binding to and activating estrogen, androgen and other hormone receptors [McKinlay et al,2008; Mnif et al,2011]. Malathion was found to inhibit catecholamine secretion and bind to thyroid

hormone receptors [Ishihara et al, 2003]. Other general toxicities of malathion at the cellular and organ level have also been identified by *in vitro* methods [Jira et al, 2012].

In addition, malathion has been reported to affect male reproductive system and spermatogenesis in animals [Choudhary et al, 2008]. Treatment with malathion decreased the body weight of earthworms' and the spermatic viability in spermatheca [Espinoza-Navarro and Bustos-Obregón, 2005]. Studies have suggested that endocrine disruptor pesticides can interfere reproductive and sexual development [McKinlay et al, 2008; Mnif et al, 2011]. Malathion impaired steroidogenesis, induced apoptosis in germ cells with proliferation of the seminiferous epithelium [Penna-Videau et al, 2012]. A recent study showed malathion induced lower testosterone level, inhibited acetylcholinesterase, and decreased reproductive performance in male mice [Selmi et al, 2014]. Exposure to malathion at the pubertal age led to alteration of semen parameters [Selmi et al, 2014]. Furthermore, malathion can affect late stages of spermatogenic cells maturation in mice causing damaged DNA and reduced chromatin in spermatogonia and spermatids [Ojha and Srivastava, 2014]. However, the full spectrum of the reproductive effects of malathion has not been fully elucidated. This study aimed to investigate the toxicity of malathion on testes in rats. We exposed the rats with different concentrations of malathion for 60 days and then investigated the activities of testicular enzymes, examined the histological changes in testis and sperm quality, and measured the hormone levels including luteinizing hormone (LH), follicle-stimulating hormone (FSH) and testosterone (T) using

radioimmunoassay (RIA). We further assessed the expression levels of genes related to reproduction.

2. Materials and methods

2.1. Chemicals

Malathion (95% pure) was obtained from WanDuoFu Chemical (Shandong, China). Malathion was dissolved in distilled water (solubility in water: 145 mg/L at 25oC) before use. RIA (Radioimmunoassay) kits for luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone (T), and assay kits for testicular enzymes including acid phosphatase (ACP), lactate dehydrogenase (LDH), succinate dehydrogenase (SDH), γ-glutamyl transpeptidase (γ-GT) were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). TdT-mediated dUTP digoxigenin nick end labeling (TUNEL) kit was purchased from Roche R&D Centre (China) Ltd. (Shanghai, China). Bax and Bcl-2 anti-rat monoclonal antibody was purchased from Santa Cruz Biotechnology (Gene Company Ltd. Beijing, China) and immunohistochemistry strept avidin-biotin complex (SABC) kit was purchased from Wuhan Boster Biological Technology. Ltd. (Wuhan, China). All other chemicals were of analytical grade and obtained from local commercial sources.

2.2. Animals and experimental design and treatment

Male Wistar rats of SPF (Specific Pathogen Free) quality weighing between 80 to 100 g were purchased from Laboratory Animal Centre of Shandong University (Shandong, China). All animals were housed in an animal room with the temperature (20 ^{o}C ± 2 ^{o}C) and relative humidity (55 % ± 5 %). Lighting period was maintained at 12h/12h light and dark cycle. Food (manufactured by Laboratory Animal Centre of Shandong Province, Shandong, China) and tap water were provided ad libitum. After 1 week of acclimatization, 40 rats were randomly divided into four groups including three exposure groups and a control group of 10 animals each. In the exposure groups, the rats were exposed to malathion by oral gavage at dosages of 33.75, 54, and 108 mg/kg daily for 60 days. Selection of dosage range was based on our pilot study for 2 weeks in which the LD_{50} of malathion is 1080 mg/kg. The control rats were administered with an equivalent volume of distilled water in the same manner. Throughout the experimental period, all animals were observed at least once daily for clinical signs of toxicity related to malathion exposure. Animals were sacrificed 60 days post dosing. Handling of animals strictly followed the ethical guidelines [Couto , 2011] in accordance with the institutional Animal Ethics Committee approval.

2.3. Physiological assessment

Body weights of rats were recorded weekly during the study and upon animal euthanasia. Then the testes were collected and weighed.

2.4. Sperm evaluation

At necropsy, the left epididymidis of each rat was collected, and the caudal epididymis was used to prepare sperm suspension for measuring sperm counts, motility, and dysmorphology rate based on the method described by Prasad, et al. [Prasad et al, 1972]. Briefly, immediately after the sacrifice, the left caudal epididymis of each animal was homogenized in 2 mL of warm (37°C) PBS solution of which 20 µL was used for the evaluation of sperm mobility. The motility of spermatozoa is measured in terms of the percentage of motile spermatozoa in total spermatozoa which was determined using a hemocytometer. The sperm smear slides were made and stained with 2% eosin for examination of the morphological changes in sperms, for which a total of 200 sperm were scored under the microscope in 100 × magnifications. The dysmorphology rates were scored and expressed as the percentage of abnormal sperm with respect to sperm from the control group.

2.5. Hormone measurement

Blood samples (3,000 µL) were taken from the retinal vein. After standing for 1 h, the blood samples were centrifuged for 10 min at 3000 rpm (1600 g), and the serum samples were stored at -70℃ until analysis. Serum LH, FSH and T levels were measured using RIA kits according to the manufacturer's protocols.

2.6. Detection of enzyme activity

Left testis tissues in 0.9 % saline buffer were homogenized by a high-speed homogenizer (FSH-2A) and centrifuged at 10,000 rpm (5333 g) at 4℃ for 30 min. The samples were stored at 4℃ until analysis. Activities of enzymes (ACP、LDH、SDH、γ-GT) were measured according to the manufacturer's protocols.

2.7. Histopathological examination

The right testis tissues were fixed in Bouin's solution and then embedded in paraffin. The paraffin-embedded sections (5 μm) were prepared and deparaffinised by 100% xylene, followed by rehydration with 100% then 70% ethanol. Transverse sections of testes were stained with hematoxylin and eosin (HE) for light microscopic examination.

2.8. Cell apoptosis detection using TdT-mediated dUTP digoxigenin nick end labeling (TUNEL) assay

Transverse sections of testis of rats from control and experimental groups were prepared using the method described above. The apoptotic cells were detected using a TUNEL assay kit (*Roche* R&D Centre (*China*) Ltd, Shanghai, China). The images were examined and captured using a digital camera attached to a fluorescent microscope (Leica DM4000B). The numbers of

apoptotic cells were quantified in 10 seminiferous tubules randomly selected from each slide and analyzed using the Leica Qwin V3 software. The apoptosis rate was presented as the percentage of apoptotic cell in total spermatogenic cells.

2.9. Immunohistochemistry

The testes were fixed, paraffin-embedded and sectioned at 5 μm thick slides as described above. Immunohistochemistry was conducted according to SABC kits instructions. Briefly, after being processed for antigen retrieval with 3% H_2O_2 treatment and 5% BSA blockage, the transverse testis sections were incubated with primary antibodies, Bax and Bcl-2 for overnight at 4°C. Horseradish peroxidase (HRP)-conjugated secondary antibody was used at a 1:700 in blocking solution for 1 h at 37°C. 3,3-Diaminobenzidine tetrahydrochloride (DAB) was used to visualize the antigens and hematoxylin (Vector, Houston, Texas) was used to counterstain the nuclei. Ten seminiferous tubules were randomly selected from each slide for light microscopic examination. Bax and Bcl-2 protein expression positive cells were quantified and analyzed using the Leica Qwin V3 software.

2.10. Statistical Analysis

Difference between the control and treated groups were evaluated statistically (Statistical Product and Service Solutions 17.0, SPSS, China) using one-way ANOVA. The data were expressed as the mean ± standard deviation (SD). Significance was set at $P<0.05$.

3. Results

3.1. Body and testis weights changed by malathion treatment

No deaths or clinical signs of toxicity were observed in rats treated with malathion at all doses for 60 days. The testes appeared normal in control group and 33.75 mg/kg treated group while rats in 54 and 108 mg/kg groups appeared to be small and mauve in colour. The body and testis weights decreased with increasing dosage of malathion. The final body weight and body weight changes significantly decreased in rats treated with 108 mg/kg malathion ($P<0.05$) and the absolute testis weight showed a statistical difference between the treated groups (54 and 108 mg/kg) and the control group ($P<0.01$) (Table A).

3.2. Malathion reduced the sperm count and motility and increased the sperm dysmorphology rates

Sperm count and motility in the caudal epididymidis of rats exposed to malathion were lower than that in the control rats. As shown in Table B, statistical differences were found in rats treated with higher concentration of malathion (108 mg/kg) when compared with that in the control group ($P <0.01$). The rates of sperm dysmorphology in rats from 54 and 108 mg/kg groups were statistically higher than that in the control rats ($P<0.01$). There were no significant differences between the 33.75 mg/kg group and control group.

3.3. Hormone levels decreased in rats exposed to malathion

Hormone levels decreased in the treatment groups as shown in Table B. Rats treated with 108 mg/kg of malathion showed significant decreases of LH, FSH and T compared with that in the control group ($P<0.01$).

3.4. Testicular enzyme activities

The results indicated that the activities of ACP and γ-GT of rats in the 108 mg/kg group were significantly lower than that in the control group as shown in Table B ($P<0.01$). Activities of LDH were significantly higher in the same treated-rats compared to the control rats ($P<0.01$). There were no significant differences in the activities of SDH between any of the malathion treated groups and the control group.

3.5. Histopathological changes induced by malathion

Histological examination showed malathion induced a dose-dependent effect on spermatogenesis of rats at all treatments. Rats from the control group showed a normal process of spermatogenesis with a regular arrangement of spermatogenic epithelium in the seminiferous tubules (Fig.A.1). Rats treated with malathion at 33.75 mg/kg showed mild testicular changes (Fig.A.2). Malathion dosed at 54 and 108 mg/kg resulted in severe alterations in the seminiferous tubules including the loss, derangement and sloughing of the spermatogenic cells, vacuolization

in Sertoli cell cytoplasm and destruction of Sertoli cell cytoskeleton (Fig.A.3 and 4). These changes were more severe in rats from the group exposed to malathion at 108 mg/kg (Fig. A.4).

3.6. Malathion induced apoptosis in testis cells

Spermatogenic cells of rats in the control group were well arrayed in order with few apoptotic germ cells as shown in Fig.B.1. The apoptosis rate was increased significantly in malathion-treated groups compared to the control group ($P<0.01$) (Fig. C). In addition, apoptotic cells with stronger staining were found among spermatogenic cells in rats exposed to malathion (Fig. B. 2, 3, and 4).

3.7. Bax and Bcl-2 protein expression

In testicular cells of control rats, weak immunoreactivity against Bax was detected as shown in Fig.D.1. Bax positive staining was observed as coarse brown granules in the cytoplasm in spermatogenic cells of rats at 54 and 108 mg/kg treated groups (Fig. D. 2, 3, and 4). Besides, the number of Bax positive staining was increased with increasing dosage of malathion. Bcl-2 immunoreactivity was identified as fine particles in varying degrees of spermatogenic cells of the control group (Fig.E.1). In rats exposed to malathion, Bcl-2 protein particles were unevenly distributed and the number of positively-stained cells decreased when compared with the control group (Fig.E.2, 3, and 4). Relative intensities of Bax and Bcl-2 protein were analyzed in Fig. F

which showed statistically significant changes in 54 and 108 mg/kg groups ($P<0.01$ and $P<0.05$) compared to the control group.

4. DISCUSSION

Malathion is a widely used OP insecticide in agriculture and home/public gardens. People may expose to malathion through multiple pathways including oral, dermal and inhalation routes. *In vitro* and *in vivo* studies suggested that malathion has a broad spectrum of toxicities among which the reproductive effect has not been fully elucidated. Therefore, this study focused on malathion-induced toxicities in the testis of male rats. We found that administration of malathion to male rats by oral gavage resulted in adverse effects that included significant decrease in the final body weights and testis weights, sperm counts, motility, and increased rates of sperm dysmorphology. These results are consistent with the published data which showed that malathion has a disruptor effect on sperm count and decreases the percentage of motile sperm of male rats [Selmi et al, 2014]. The weight of testes is largely dependent on the mass of differentiated spermatogenic cells [Chiou et al, 2008]. The reduced tubule size, decreased number of germ cells and elongated spermatids may lead to the reduction in the weight of testes as observed in this study. Sperm quality is an important indicator for fertilizing ability. It is a sensitive index to study the effect of a variety of physical and chemical factors to reproductive

cells [Adhikary et al, 1989]. Malathion-induced the reduction in sperm quality found in this study indicates toxic effects of malathion on the reproductive system of rats. We further confirmed the effects by histological studies. Our histological examinations revealed that malathion-caused testicular lesions were characterized by markedly decreased testis weight with moderate to severe widening of interstitial spaces and arrested spermatogenesis which was more obvious in rats treated with malathion at 108 mg/kg. We also observed atrophic seminiferous tubules, disordered and decreased spermatogenic cells, increased degeneration, and sloughing of spermatogenic cells. This could indicate that malathion alters the testicular function seriously in rats similarly as reported earlier for mice treated with organophosphates [Sobarzo and Bustos Obregón , 2000].

Hormone levels and relevant enzymes have been reported to be associated with the reproductive functions [Ishihara et al, 2003; Vidair, 2004]. Therefore, we examined the serum hormone (LH, FSH and T) levels and found an inhibitory effect on these hormones by malathion. The reduction of T may be due to the adverse effect of malathion on the rat hypothalamic-pituitary-testicular axis leading to an imbalance in the testis regulation and consequent reduction in the secretion of pituitary gonadotrophins (FSH and LH). Krause and Homota [Krause and Homola , 1974] proposed that the decreased testosterone level might be due to a direct damage of Leydig cell or to lowered stimulation of these cells by interstitial cells stimulating hormone. The changing of serum hormone levels observed in this study further confirmed the deleterious

effects of malathion on the reproductive system of male rats. The activity of LDH was increased while the activities of ACP and γ-GT were decreased in rats treated with malathion at 108mg/kg group. The altered enzyme activities by malathion may be resulted from the damaged mature seminiferous epithelium and the function of sertoli cell and disrupted normal spermatogenesis and metabolism in rats.

It has been reported that OP can cause various histopathological changes in the reproduction system of rodents [Uzunhisarcikli et al, 2007]. To explore the possible mechanisms involved in malathion-induced reproductive toxicity, we investigate the apoptosis since it has been reported that malathion can induce apoptosis-related gene expression [Aboul-Soud et al, 2011]. We found that malathion can increase the rate of apoptosis in spermatogenic cells. We then confirmed the malathion-induced apoptosis by examining the apoptosis-related proteins Bax and Bcl-2 which have been shown to be expressed in rodent and human testis [Xu et al, 2000; Koji and Hishikawa, 2003]. It has been known that Bax promotes apoptosis by depleting the growth factors while Bcl-2 inhibits apoptosis by inactivating multiple proapototic proteins [Youle and Strasser, 2008]. The balance between the expression levels of the two genes determine whether the cells can survive or not [Korsmeyer, 1999; Basu and Haldar, 1998]. Data from this study clearly indicated the apoptotic effect of malathion by increased expression of Bax and decreased Bcl-2. Thus, the delayed spermatogenic cell differentiation and proliferation, the reduced number of sperm cells and spermatozoon may be attributed to the apoptosis by malathion.

5. Conclusion

Our results demonstrated reduced body and testicular weight, pathological changes in the testes and depressed activities of testicular enzymes in the rats exposed to malathion. Results from this study further confirmed the deleterious effects of malathion on the general and reproductive system in mammalian animals. The malathion-induced abnormal levels of hormones relative to reproduction and apoptotic protein levels involved in apoptosis may play important roles in these effects. Further studies to explore how malathion affect the these hormones and proteins and their cross-talk in contribution to these effects are required in order to fully elucidate its mode of action. These studies will provide important information about prevention strategy for reducing potential hazardous effects of malathion.

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Tables

Table A: Body and absolute testis weight (g) of male rats exposed to malathion

Parameters	0(control) (n=10)	33.75 mg/kg (n=10)	54 mg/kg (n=10)	108 mg/kg (n=10)
Initial body weight (g)	116.30±8.92	114.40±7.06	114.80±5.47	118.20±6.43
Final body weight (g)	402.10±33.36	380.40±54.26	345.50±51.16	334.90±53.00*
Body weight change (g)	285.80±28.95	266.00±53.51	230.70±51.53	216.70±55.92*
Absolute testis weight (g)	4.26±0.42	3.80±0.25	3.43±0.37**	3.36±0.81**

Results are presented as means±SD.

* Indicate a significant difference at $P<0.05$ compared with the control group;

**Indicate a significant difference at $P<0.01$ compared with the control group.

Table B: Sperm characteristics, hormone levels and testicular enzyme activities of male rats exposed to malathion

Parameters	**0(control) (n=10)**	**33.75mg/kg (n=10)**	**54mg/kg (n=10)**	**108mg/kg (n=10)**
Sperm characteristics				
Sperm counts (10^9)	9.26±2.15	9.19±2.05	7.36±1.93	5.54±3.12**
Sperm motility (%)	78.50±11.94	73.70±12.28	41.60±13.37**	36.10±13.37**
Dysmorphology rates (%)	0.24±0.06	0.37±0.09	0.57±0.08**	0.63±0.13**

Hormone levels				
LH (mIU/ml)	1.08±0.47	0.73±0.39	0.52±0.34**	0.49±0.19**
FSH (mIU/ml)	2.05±0.50	1.79±0.53	1.53±0.68	1.25±0.31**
T(nmol/L)	3.35±1.20	2.94±0.82	2.67±1.08	1.91±1.27*
Testicular enzyme activities				
ACP (U/g Pro)	1.46±0.31	1.31±0.11	1.20±0.27	1.13±0.21**
LDH (U/g Pro)	0.19±0.11	0.24±0.04	0.25±0.11	0.31±0.05**
SDH (U/mg Pro)	96.81±16.01	100.21±19.67	84.42±21.58	87.36±20.66
γ-GT (U/g Pro)	1.22±0.12	1.19±0.13	0.95±0.13**	0.82±0.21**

Results are presented as means±SD.

* Indicate a significant difference at $P<0.05$ compared with the control group;

**Indicate a significant difference at $P<0.01$ compared with the control group.

Conflict of interest statement

The authors declare that they have no competing interests.

Figure legends

Fig.A. Histological changes in the testes of rats exposed to malathion. 1: Control; 2: 33.75 mg/kg; 3: 54mg/kg, 4: 108 mg/kg. The transverse sections of testes were stained with hematoxylin and eosin (HE). The images were captured by a digital camera attached to a fluorescent microscope (Leica DM4000B) at × 400 magnification.

Fig.B. Malathion-induced cells apoptosis in rat testes. TUNEL assay was conducted to detect the apoptosis cells in the testes of rats from control group (1) and malathion treatment at 33.75 mg/kg (2), 54 mg/kg (3) and 108 mg/kg (4) for 60 days. The images were captured by a digital camera attached to a fluorescent microscope (Leica DM4000B) at × 400 magnification.

Fig.C. Apoptosis rate in spermatogenic cells of male rats exposed to malathion. The numbers of apoptotic cells were quantified in 10 seminiferous tubules randomly selected from each slide and analysed using the Leica Qwin V3 software. The apoptosis rate is measured in terms of the percentage of apoptotic cells in total spermatogenic cells. $^{**}P<0.01$.

Fig.D. Bax expression in the rat testes after exposed to malathion. Immunohistochemistry staining using a specific antibody against Bax in testes of rats from control (1) and malathion at concentrations of 33.75 mg/kg (2), 54mg/kg (3), 108 mg/kg (4). The Bax positive staining was observed as coarse brown granules in the cytoplasm in spermatogenic cells of rats from

experimental groups treated with malathion at 54 and 108 mg/kg. The photomicrographs were taken at × 400 magnification.

Fig.E. Bcl-2 expression in the rat testes after exposed to malathion. Immunohistochemistry staining using a specific antibody against Bax in testes of rats from control (1) and malathion at concentrations of 33.75 mg/kg (2), 54mg/kg (3), 108 mg/kg (4). The photomicrographs were taken at × 400 magnification.

Fig.F. Relative intensities of Bax and Bcl-2 protein expression in testis cells of male rats exposed to malathion. Relative intensities were expressed as the the percentage of treatment group in control group. **P*<0.05, ***P*<0.01.

malathion at 54 and 108 mg/kg. The photomicrographs were taken at × 400 magnification.

Fig.E. Bcl-2 expression in the rat testes after exposed to malathion. Immunohistochemistry staining using a specific antibody against Bax in testes of rats from control (1) and malathion at concentrations of 33.75 mg/kg (2), 54mg/kg (3), 108 mg/kg (4). The photomicrographs were taken at × 400 magnification.

Fig.F. Relative intensities of Bax and Bcl-2 protein expression in testis cells of male rats exposed to malathion. Relative intensities were expressed as the the percentage of treatment group in control group. *P<0.05, **P<0.01.

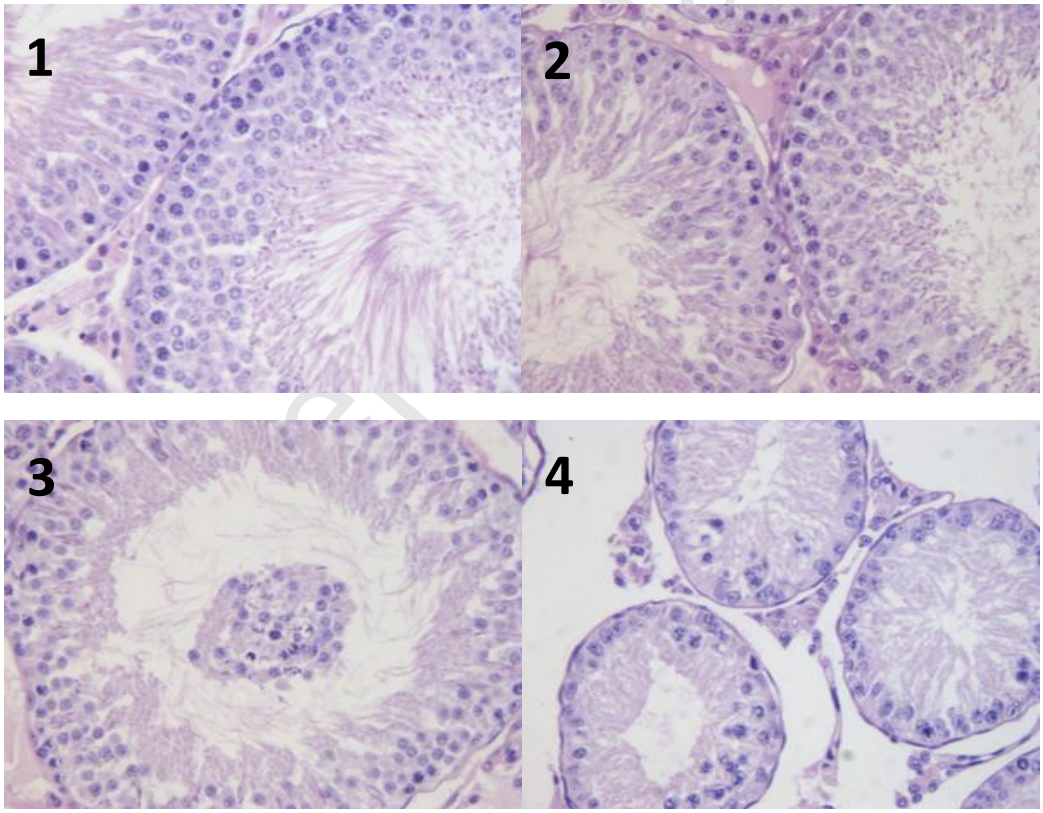


Fig. A

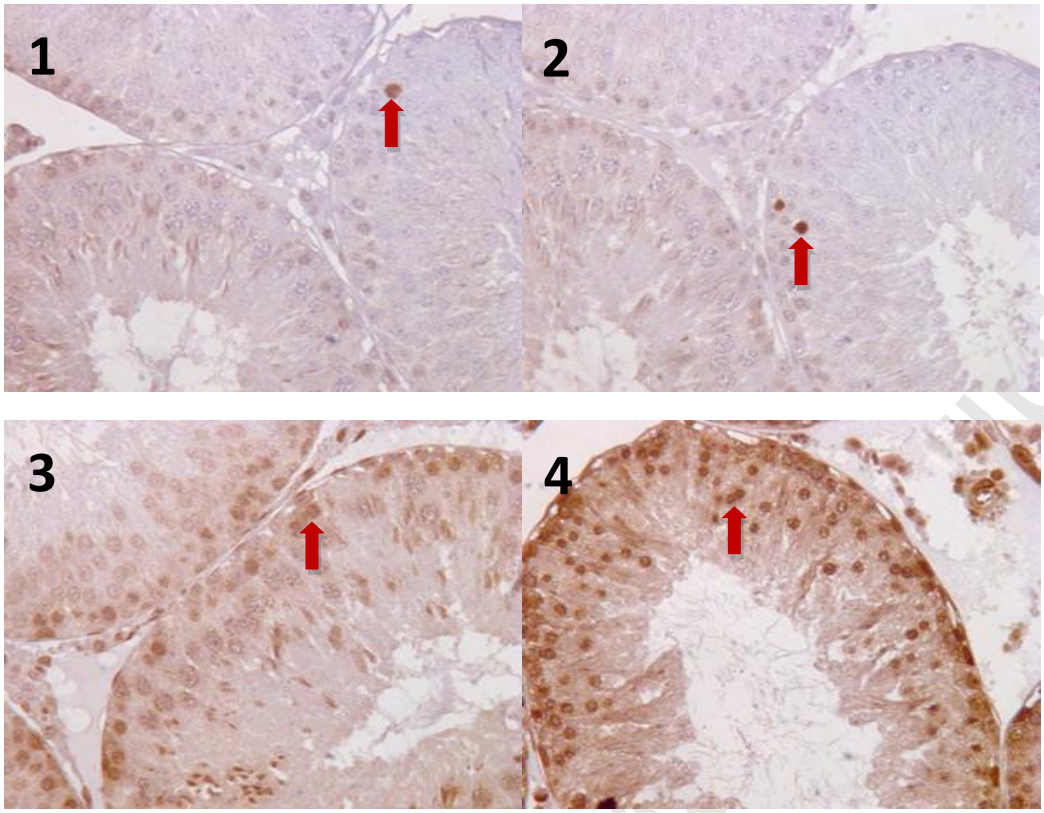
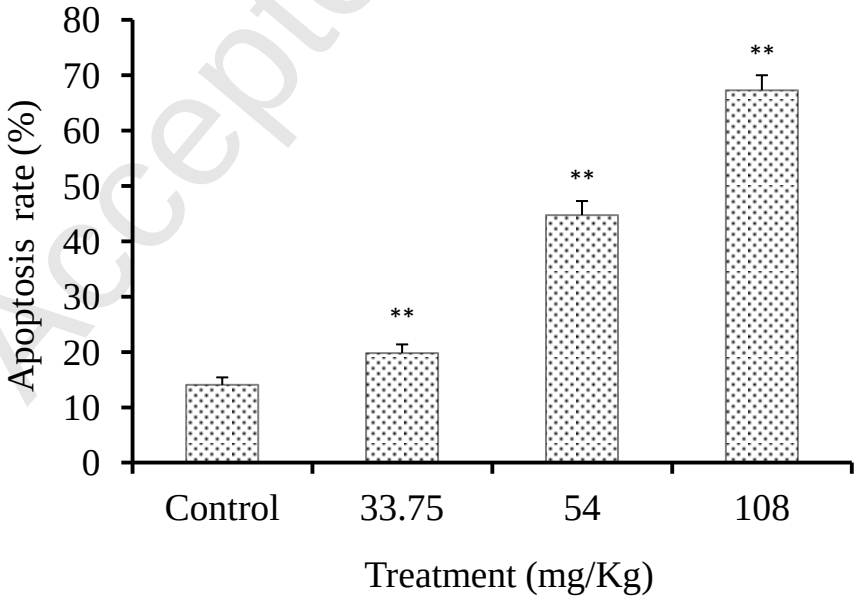


Fig. B



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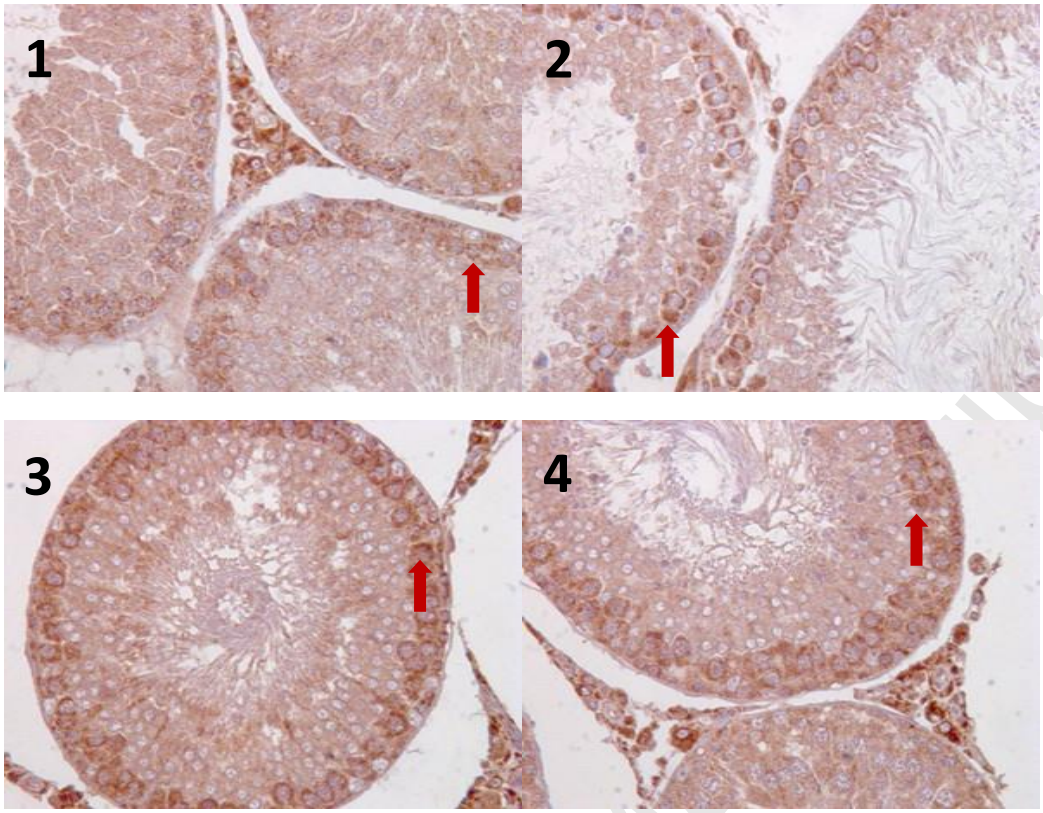

Fig. D

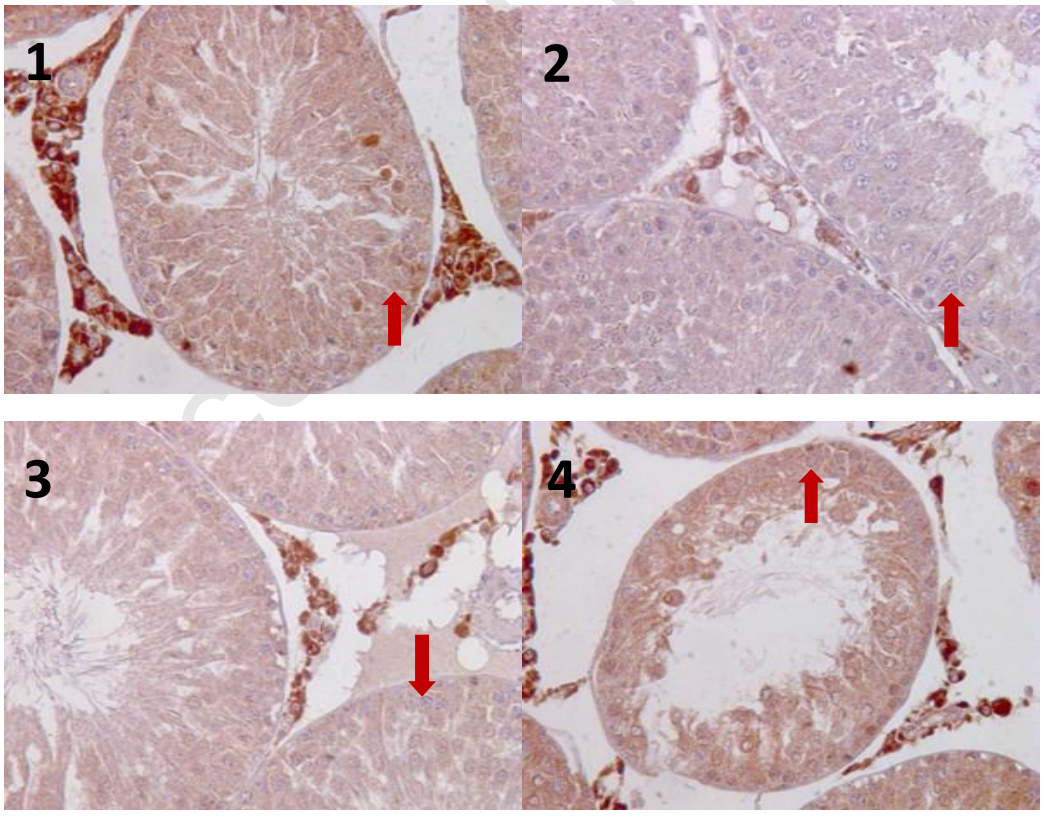

Fig. E

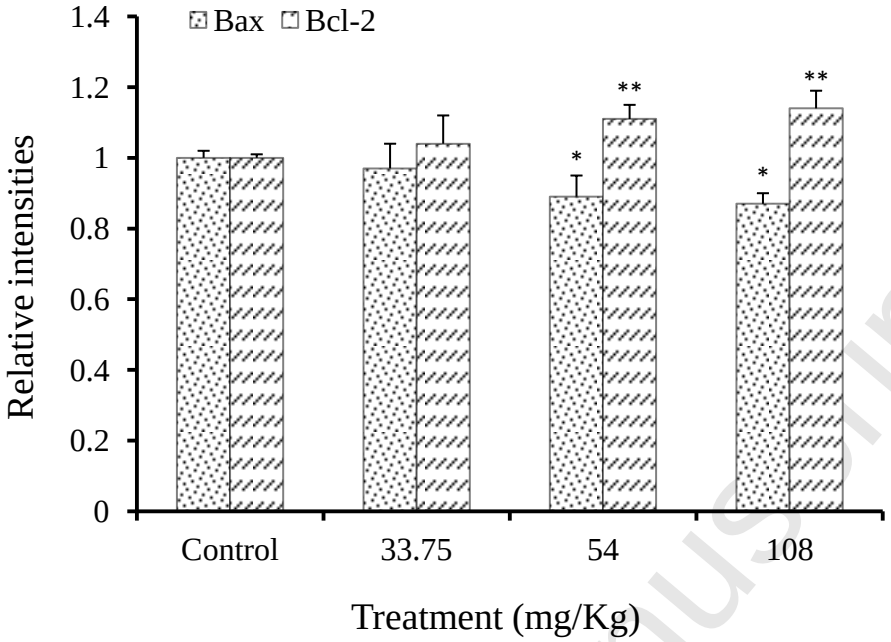

Fig. F

Tables

Table A: Body and absolute testis weight (g) of male rats exposed to malathion

Parameters	0(control) (n=10)	33.75 mg/kg (n=10)	54 mg/kg (n=10)	108 mg/kg (n=10)
Initial body weight (g)	116.30±8.92	114.40±7.06	114.80±5.47	118.20±6.43
Final body weight (g)	402.10±33.36	380.40±54.26	345.50±51.16	334.90±53.00*
Body weight change (g)	285.80±28.95	266.00±53.51	230.70±51.53	216.70±55.92*
Absolute testis weight (g)	4.26±0.42	3.80±0.25	3.43±0.37**	3.36±0.81**

Results are presented as means±SD.

* Indicate a significant difference at $P<0.05$ compared with the control group;

**Indicate a significant difference at $P<0.01$ compared with the control group.

Table B: Sperm characteristics, hormone levels and testicular enzyme activities of male rats exposed to malathion

Parameters	**0(control) (n=10)**	**33.75mg/kg (n=10)**	**54mg/kg (n=10)**	**108mg/kg (n=10)**
Sperm characteristics				
Sperm counts (10^9)	9.26±2.15	9.19±2.05	7.36±1.93	5.54±3.12**
Sperm motility (%)	78.50±11.94	73.70±12.28	41.60±13.37**	36.10±13.37**
Dysmorphology rates (%)	0.24±0.06	0.37±0.09	0.57±0.08**	0.63±0.13**
Hormone levels				
LH (mIU/ml)	1.08±0.47	0.73±0.39	0.52±0.34**	0.49±0.19**
FSH (mIU/ml)	2.05±0.50	1.79±0.53	1.53±0.68	1.25±0.31**
T(nmol/L)	3.35±1.20	2.94±0.82	2.67±1.08	1.91±1.27*
Testicular enzyme activities				
ACP (U/g Pro)	1.46±0.31	1.31±0.11	1.20±0.27	1.13±0.21**
LDH (U/g Pro)	0.19±0.11	0.24±0.04	0.25±0.11	0.31±0.05**
SDH (U/mg Pro)	96.81±16.01	100.21±19.67	84.42±21.58	87.36±20.66
γ-GT (U/g Pro)	1.22±0.12	1.19±0.13	0.95±0.13**	0.82±0.21**

Results are presented as means±SD.

* Indicate a significant difference at $P<0.05$ compared with the control group;

**Indicate a significant difference at $P<0.01$ compared with the control group.