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THE EFFECT OF METHIDATHION ON SEX HORMONES, SPERM PARAMETERS AND TESTICULAR TISSUE IN MICE

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ABSTRACT

The present study aimed to identify the effect of methidathion on sex hormones, sperm parameters and testicular tissue in mice *Mus musculus*. Adult male mice divided into three groups (8 for each group), the first group i.p injected with normal saline as a control group, the second and third groups were i.p injected with (1.2, 2.4mg/kg of body weight) methidathion respectively for 15 day. The level of LH wasreduced in mice given high dose methidathion and decrease levels of testosterone in low and high doses compared with control group.A significant decreased in number of sperm after methidathion injection and significant increased in proportion of malformation sperm rate shown in high dose compared with control group. histopathological examination of testes showed analysis in some seminiferous tubules.

KEYWORDS: methidathion, histopathology, sperm, hormones.

INTRODUCTION

Environmental pollution is one of the most important problems facing humans in the modern times which results from the addition of different chemical substances to the environment, that were developed by humans and which were not previously known, such as pesticides of various kinds, agricultural fertilizers, types of plastics and industrial detergents.[1]

Pesticides are the most dangerous environmentalcontaminants that affect ecological balance due to the adverse effects of non-target organisms[2], pesticide are most important technological inputs in agricultural production that reduce losses caused by agricultural pests.[3]

The importance of pesticide is to increase production by affecting pests that damage 35% of all food crops before harvest[4], classification of pesticides based on chemical composition into several types: Organochlorine, Organophosphates, Carbamates, Pyrethroids and others.[5]

Organophosphates (OP) pesticide are widely used in agriculture, industry and public health due to their high insecticidal activity, low environmental stability and moderate toxicity.[6] However, the frequent use of OP pesticide and accessibility have increased their risk.[7]

MethidathionS-(2,3-Dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol-3-ylmethyl) O,O-dimethyl phosphorodithioate, is one of the most widely OP pesticide used in the world for pest control of crop[8], The department of pesticide regulation (DPR) Environmental Protection Agency placed methidathion on high-priority roster for risk and its value based on the conceivable adverse effects in chronic toxicity, carcinogenesis and chromosomal anomalies.[9]

Methidathion and the substances produced by the metabolite within the body of organism their toxic reaction primarily through their irreversible inhibition of cholinesterase (ChE) enzymes[10,9], the main way to metabolize methidathion in liver was through glutathion-s-transferase and the primary metabolizer was desmethylmethidathion.[11]

Exposure to methidathion can cause toxic effects on various body systems such as liver, kidney and heart[12,13;14], methidathion have also been reported to affect the reproductive system in female.[15] However, the pathophysiological effect of methidathion on sperm and testes remains unclear and underreported. While several studies have studied other phosphorus pesticides,[16] studied the effect of malathion on rats with a concentration of 27mg/kg; 1/50 Ld50 The results showed a decrease in the number of sperm count and the movement of sperm motility and levels of LH, FSH, and testosteronal hormones decreased, The histopathological changes were characterized by the presence of necrosis and edema in seminiferous tubulesand interstitial tissue.

The aim of this study is to determine the effect of methidathion exposure on the reproductive system in male mice with two dose by injection intraperitoneal of mice.

MATERIALS AND METHODS

Chemicals

Methidathion (Ultracidin) solution concentrated pack size 1L containing methidathion with 40%. Produced by FABCO-Jordan used commercially and purchased from local markets.

Experimental animals and treatment protocol

Male mice aged (10-12) weeks, and weight (20-25)g were used in the study. The mice were obtained from Department of Biology, College of Education for Pure Sciences, University of Basrah, Iraq. They were residence in a room at constant temperature ranging from 20-25^{0}C with 12 h light/ dark cycles[17], the mice were placed in standard sizeplastic cages (30× 10×11cm) manufactured by a company North Kent plastic Kent U.K, the cages were supplied with a log of wood that was changed weekly, and fedingintegrated diet.

The mice were randomly divided intothree groups with eight animals in each as follows:

1. The first group (control): were intraperitoneally (i.p) injected with 0.1 ml of normal saline.
2. The second group (low dose): were i.p injected with 0.1 ml of 1.2 mg/kg of methidathion.
3. The third group (high dose): were i.p injected with 0.1 ml of 2.4 mg/kg of methidathion. All group were daily injected for a period of 15 days.

Serum analysis

At the end of the injection period (15 day), the animals were sacrificed, blood was collected directly from heart of each animal into gel tubes, at room temperature for clotting. Serum was by centrifuged at 3000 rpm. for 30min and analyzed, The levels of follicle stimulating hormone (FSH), luteinizing hormone(LH), and testosterone were assayed by kits were used (monobindInc. lake forest CA92630, USA).

Sperm count

Vega *et al*. 's method[18] was used in sperm count by taking the right epididymis of rat and cut into very small parts in one milliliter of phosphate buffered saline (PBS, PH=7.2).

Sperm maliformations

Wyrobek and Bruce 's method[19] was used for the purpose of the study of maliformations of sperm by taking the left epididymis and cut into very small pieces in a petri dish that contains 5 ml of phosphate buffered saline (PBS).

Histopathological examinations

The testes were isolated after the animals were killed and they were immediately fixed in Bouins solution for 24hr after routine processing, paraffinsections were cut into (5µm)

thickness. These section were stained by hematoxylin-eosin (H&E)[20], and morphometrically examined by light microscopy.

Statistical analysis

The Results are analyzed statistically by the Analysis of variance (ANOVA-R.L.S.D) test. The results are expressed as Mean± S.E. the statisticalpackage for the social sciences (SPSS, V.11). Significance was set at the level of P≤ 0.05.

RESULTS

Plasma levels of FSH, LH, and testosterone

The high dose had significantly reduced plasma LH concentration compared to the control group (P≤ 0.05). While the results did not show a significant difference in the level of hormone FSH between all groups (P≤ 0.05). The results indicated a significant decreased in testosterone level in mice given the low and high doses compared with control group (P≤ 0.05), while it has not shown any significant difference between the two doses in testosterone level (table 1).

Evaluation of testicular sperm count

The results showed a significant decrease (P≤ 0.05) in spermnumber of the group given low and high dose compared with the control group, significant difference (P≤ 0.05) shown between the two doses (table 2).

Evaluation of sperm malformations

The study showed a significant decrease (P≤ 0.05) in percentage of normal sperms in high dose and significant increases in percentage of malformationsrate sperm heads and tails in high dose compared with control group and showed significant difference between the two doses (table 2).

Histopathological changes in testis

Figure 1A-1D shows testes from the different experimental groups. Normal testicular histology was observed in control group (Figure 1A). Testes of the low-dose methidathion group showed a slight changes from the normal histological features with decrease layers of the wall seminiferous tubule resulted from lack of primary and secondary spermatocyte and disappearance of spermatids in some parts of the tubules (Figure 1B). Testicular sections of

high –dose group showed various stages of the decomposition or complete analysis of wall seminiferous tubule, lumen filled with cellular debris and is devoid of sperm (Figure 1C, D).

Table: 1 Effect of methidathion on sex hormone of male mice

Parameters Groups	FSH	LH	Testosterone
Control (normal saline)	1.011±0.485	[a]3.525±1.179	[a]3.361±0.160
low dose 1.2 mg/kg	1.120±0.256	[a]3.392±0.883	[b]1.502±0.535
high dose2.4 mg/kg	2.136±0.468	[b]0.147±0.016	[b]1.018±0.090
R.L.S.D	N.S	2.41	0.892

Values are means ± S.E., Different letters refer to a significant difference at (P≤0.05).

N.S refer to non-significant differences at (P≤0.05).

Table: (2) Effect of methidathion on sperm of male mice

Parameters Groups	The sperm count ×106	normal sperm	Sperm maliformations	
			Head	tail
Control (normal saline)	a9.387±0.665	a88.875±1.913	b4.25±0.890	b6.875±1.112
low dose 1.2 mg/kg	b6.293±0.647	a80.875±2.470	b6.218±0.651	b12.906±2.008
high dose2.4 mg/kg	c3.400±0.635	b56.125±4.674	a10.625±1.934	a33.250±4.408
R.L.S.D	1.699	8.493	3.654	7.507

Values are means ± S.E., Different letters refer to a significant difference at (P≤0.05).

N.S refer to non-significant differences at (P≤0.05).

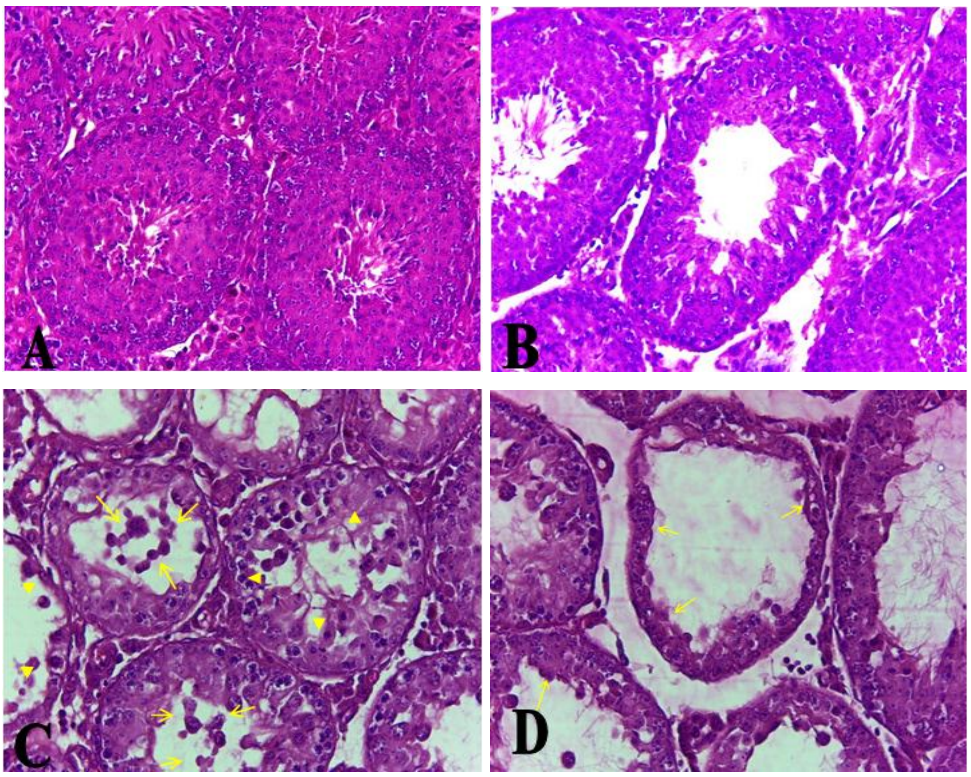

Figure: 1A. Optical microscopy images of mouse testis in control group staining with (H& E 400×). Figure 1B.the transverse section of the testis from a treated mouse with

methidathion (1.2 mg/kg) showdecrease layers of the wall seminiferous tubule resulted from lack of primary and secondary spermatocyte and disappearance of spermatids in some parts of the tubules (H&E 400X), Figure 1C. the transverse section of the testis from a treated mouse with methidathion (2.4 mg/kg) showed various stages of the decomposition of wall seminiferous tubule(head arrows) , lumen filled with cellular debris and did not contain sperm (arrows) (H&E 400X), Figure 1D.the transverse section of the testis from a treated mouse with methidathion (2.4 mg/kg) showed advanced stage of decomposition of wall seminiferous tubule, complete analysis of spermatids and secondary spermatocyte and a few numbers from primary spermatocyte (arrows) (H&E 400X).

DISCUSSION

Exposure to environmental pollutants leads to affect testicular function by reduction pituitary LH secretion and reducing leydig cell steroidogenesis[21,22], Organophosphates (OP) pesticide depression serum steroid hormone levels by elevate catabolism and elimination or directly inhibit steroid hormone production.[23] In the current study, the exposure of mice to methidathion caused a reduction LH level and testosterone. Our results are in agreement with the findings of Maitra and Mitra[24] who reported a reduction in LH level and testosterone after exposure of birds to methyl parathion. While another study found that pesticide Methomyl elevate FSH and LH Levels [25]. [26] show the ability of chronic Chlorpyrifos exposure to induce oxidative damage to the pituitary gland and testes, and they concluded oxidative stress to these organs may be partly responsible for the testicular and reproductive dysfunctions. Pesticide are hydrophobic molecules that bind widely to biological membranes, essentially phospholipid bilayers[27], and they may cause damage to the membranes by generate lipid peroxidation.[26]

Previous studies have reported that methidathion caused oxidative damage by lipid peroxidation in rat aortic wall, heart tissue, liver and ovary[28,12,8,15].[29] have shown that single-dose treatment with methidathion increases lipid peroxidation in rat erythrocytes.[8] have shown lipid peroxidation may be a molecular mechanism contributory in methidathion induced toxicity. lipid peroxidation occur through the generation of reactive oxygen species (ROS) that may be produced as a result of methidathion metabolism or due to high energy consumption to inhibit oxidative phosphorylation.[30]

[31]showed that induction abnormal sperms was assumed to be a result of a direct effect of diazinon on testicular tissue in rat, as Pina-Guzman *et al*.[32] proposed DNA as a target of organophosphates, although the installation of DNA is tight packaging, and the antioxidant present in seminal plasma. However, oxidative stress may manifest as a mediator between reactive oxygen species (ROS) generation and scavenging antioxidants[33]. [34] reported evidence of increased signs of oxidative stress, such as malondialdehyde and decreased antioxidant enzyme in the testis after exposure fenitrothion in male spraguedawley rats. As well as spermatozoa are particularly vulnerable to oxidative stress because of their high content of polyunsaturated fatty acids (PUFAs) in their plasma membranes and their cytoplasm contains low concentration of scavenging antioxidants[35], which causes abnormal sperm after exposure Organophosphates

The decrease in the number of sperm in the present study may be due to low level of hormone testosterone, [36]who explained that maintenance of testosterone levels very important in the process spermatogenesis and fertility. The damaged Sertoli cells in present study caused decrease in the number of sperm, As[37] indicated the toxic substances have a direct effect on the function of theSertoli cells and they are involved in controlling the spermiation, and when disturbed caused epithelial disorganization and following tubular atrophy. the results of the current study are in concurred with[38] who showed that decrease in sperm count and serum testosterone after exposure to deltamethrin in male rat for 28 days. As a results of The tissue damage in the testis tissue caused by the pesticide may occur due to the direct effect of pesticide on the cell or tissue or can occur due to the levels of the imbalanced hormone.[39]

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