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EFFECT OF LONG-TIME MALATHION ADMINISTRATION ON TESTOSTERONE, OXIDATIVE STRESS, AND SPERM CHARACTERISTICS IN RATS

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ABSTRACT

Organophosphorus pesticides are one of the most extensively used forms of pesticides that could adversely impact fertility in both animals and humans. The current research aimed to investigate the potential effects of long-time malathion administration on testosterone in serum, oxidative stress biomarkers in testicular tissue and sperm characteristics of rats as an experimental model. Twenty adult male Wistar rats were split into two groups: the control group (n = 10) and the malathion-treated group (n = 10) based on the medication received orally by gavage (3 times/week for 60 days). Long-time administration of malathion negatively affected testosterone level, sperm count, sperm viability, and sperm morphology when compared to the control group. Lipid peroxidation increased significantly in the malathion-treated group when compared to the control group. On the other hand, Malathion administration caused a significant reduction in testosterone level, activities of glutathione peroxidase, reduced glutathione, and superoxide dismutase enzymes in the testicular tissue of male rats. In conclusion, long-term with space interval administration of malathion had deleterious effects on testosterone level and testicular oxidative status as well as semen quality in male rats. Consequently, it is essential to monitor surveys of organophosphorus pesticide residues in plants to protect consumer health.

***Keywords**:* Malathion; oxidative status; semen quality; testes; and male rats.

INTRODUCTION

Hazardous pesticides' widespread use and toxicological effects have a negative impact on a variety of environmental factors, including animals and humans (Mohamed *et al.*, 2022). These effects might be directly observed through bioaccumulation or indirectly via the food chain. Therefore, extremely poisonous pesticides pose a threat to both animal and human health and the environment due to their ability to inhibit enzyme activity and cause oxidative stress (Parra-Arroyo *et al.*, 2022). Insect resistance

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to conventional pesticides is a major issue that has cost billions of dollars in agriculture and could spread many insect-borne diseases (Gould *et al.,* 2018).

Organophosphorus compounds (OPs) are one of the most extensively used forms of pesticides. They play an essential role during the production process due to their effects on avoiding pathogen infection and enhancing output. However, in the early stages of the development and implementation of OPs. The long-term utilization of OPs has raised concerns about their effects on public health (Fu *et al.*, 2022).

Malathion is one of the world's first organophosphate insecticides, and it is still widely used in Egypt, mostly for agricultural purposes (el Okle *et al.,* 2022). The indiscriminate and excessive use of pesticides, especially the common ones in Egypt such as malathion in the developing villages, causes many problems that directly negatively affect human health and indirectly through animal byproducts that humans feed on, even those that are frozen for long periods (Hassouba, M. *et al.,* 2007). Even at low dosages, persistent malathion exposure is frequently associated with severe hepatic, renal, and testes problems in laboratory animals (Badr, 2020; Omar *et al.,* 2022a), due to it has been demonstrated that malathion intoxication increases oxidative stress as a result of excessive production of reactive oxygen species (ROS) and exhaustion of the body's natural antioxidant defense system, which interact with biomolecules to cause lipid peroxidation, numerous biochemical inactivation, and DNA damage (Ibrahim *et al.,* 2020). (EL ASUOTY, M.S *et al.,* 2017) reported that farm animals eating feed and grains exposed to malathion can transfer it to milk and its derivatives. As a result, continuous monitoring of pesticide residues in feed, whether for humans or animals, particularly dairy animals, is critical to protecting consumer health and achieving food safety.

Malathion intoxication has been previously shown to be implicated in the disruption of animal sexual and reproductive development (Espinoza-Navarro & Bustos-Obregón, 2005; Geng *et al.,* 2015; Kara & Öztaş, 2021) due to malathion reported to have the ability to cross the blood-testes barrier (Uzunhisarcikli *et al.,* 2007). Malathion was found to suppress seminiferous epithelium growth while impairing steroidogenesis and inducing death in germ cells (Geng *et al.,* 2015). Further, it interferes with the differentiation of spermatogenic cells in the late stages in mice, resulting in DNA damage and decreased chromatin in spermatogonia and spermatids.

Many studies have shown that using the insecticide malathion on a daily basis and in different concentrations leads to the deterioration of fertility in animals. Furthermore, acute toxicity of malathion increases the production of oxidative stress and decreases the antioxidant when used daily for 28 days with a dose of (100 mg kg–1 day–1 by the oral route) (Akbel *et al.,* 2018), also (Ibrahim *et al.,* 2020) reported used of malathion daily for 30 days at a dose (27 mg/kg/day) via gavage inducing an increase in malondialdehyde contents and decrease serum levels of follicle stimulating hormone, luteinizing hormone, testosterone, and testicular levels and activities of glutathione, glutathione peroxidase, superoxide dismutase.

Malathion's reproductive effects have not yet been fully understood in their entirety (Ojha & Srivastava, 2014). As a result, the current study sought to evaluate the effects of long-term malathion administration with time intervals on testosterone levels, sperm characteristics, lipid peroxidation, and testicular antioxidant enzymatic activities in male rats.

MATERIALS AND METHODS

1. Ethical approval

All animal procedures were carried out in accordance with the basic guidelines for the

use of lab animals. (Osborne *et al.,* 2009) and were approved by the Faculty of Medicine Institutional Review Board (IRB 17101784) of Assiut University - Egypt.

2. Animals and housing

Twenty sexually mature male Sprague Dawley rats of SPF (Specific Pathogen Free) weighing (150.5±16.6) gm, were purchased from Assiut University's Faculty of Medicine's Laboratory Animal House. One week before the research study, each of the five rats was housed in a polypropylene cage and acclimated to laboratory conditions (temperature (26^{o}C), air humidity (40-60%), and a 12-hour daylight cycle). Feed and water were available *add libitum*, using commercial pellet rat feed 21% protein.

3. Experimental design

Rats were split into two equal groups, namely, the control group (G1, n = 10) and the Malathion group (G2, n = 10). Commercial Malathion 57% (Diethyl (Dimethoxythiophosphorylthio) succinate) was purchased from AGroChem Company, El-Behera, Egypt. Three times per week, rats in the control group G1 received 0.2 ml of corn oil via gavage, while those in the G2 received a single oral dose of malathion at 27.0 mg/kg, three times per week, dissolved in corn oil (1/50 of the LD50 for an oral dose of Malathion) (Ali & Ibrahim, 2018). Malathion was given to non-fasted rats. The study's first day was marked as day 0. At the end of the eighth week of the experiment (one spermatogenesis cycle in rats) (Perrard *et al.*, 2016), Blood samples were collected in vacutainer plain tubes, which were obtained from the medial canthus of the orbital cavity of rats and centrifuged at 4500 rpm for 30 min to extract serum for testosterone measurement. All rats were sacrificed by cervical dislocation, dissected, and the right testes were manipulated to determine the testicular oxidative stress biomarkers, while the epididymal sperm viability, morphology, and testicular sperm counts were assessed using the left testes.

4. Hormone assay:

4.1. Testosterone (T)

BioCheck, Inc., Foster City, California, provided this kit. The intra- and inter-assay coefficients of variation for testosterone were 6.4% and 8.4%, respectively. The hormone was estimated using Testosterone Enzyme Immunoassay Test Kit with a Catalog Number: BC-1115 according to Tietz, (1995).

5. Semen quality assessment:

5.1. Sperm count (%):

The epididymis was crushed, and the supernatant fluid was diluted (1:100) with a solution of 5 g NaHCO3, 1 mL formalin (35%), and 25 mg eosin per 100 mL D.W. Under a light microscope at 200 magnification, diluted semen was counted in the large five squares of the Neubaur hemocytometer (Prasad *et al.,* 1972)**.**

5.2. Sperm viability (%):

One drop of sperm sample from the cauda epididymis was added, besides the one drop of eosin solutions (2 grams of eosin Plus 100 ml of 2.9% sodium citrate), plus two drops of Nigrosin stain solution (10 grams Plus 100 ml of distilled water with boiling), and the combination was smeared on slides and air dried before being inspected under a light microscope. A living sperm has an unstained head, whereas a dead sperm has a purple to red stained head according to (World Health Organization, 2010).

5.3. Sperm morphology (%):

A drop of sperm sample was placed on a slide, let to dry, then rinsed with a mixture of four parts of methyl violet (1 gram plus 100 ml distal water) plus one part of sodium carbonate (1 gram plus 100 ml distal water), followed by filter paper cleaning and drying and observation under a light microscope. At least one hundred spermatozoa from diverse fields were investigated on each slide, morphological anomalies were counted and photographed, and the percentage was calculated and tabulated based on (Menkveld, 2010).

6. Oxidative stress biomarkers:

The testicular tissue was perfused with a PBS (phosphate buffered saline) solution with a pH of 7.4 and 0.16 mg/ml heparin to clear out any red blood cells after the left testes were dissected. For each gram of testes, 10 ml of cold buffer was used to homogenize testicular tissue (50 mM potassium phosphate, pH 7.5.1 mM EDTA), centrifuged for 15 minutes at 4,000 rpm. the supernatant was collected and kept at -20°C.

6.1.Lipid peroxidation:

MDA was evaluated by a kit that was obtained from Biodiagnostic., Egypt. MDA activity was detected according to (Ohkawa *et al.*, 1979) using the supernatant of the testicular homogenate (Colorimetric Method). For 30 minutes, Thiobarbituric acid (TBA) and MDA react in an acidic medium to produce a Thiobarbituric acid reactive product, whose absorbance was measured at 530 nm.

6.2. Glutathione peroxidase (GPx) activity:

We purchased the GPx cellular activity assay kit from a Biodiagnostic Company in Egypt. The UV Method was used to estimate GPx activity based on (Paglia & Valentine, 1967). The assay is based on the GPx-mediated oxidation of (GSH) to (GSSG), followed by glutathione reductase and NADPH-mediated recycling of GSSG back to GSH. Over the duration of three minutes, against deionized water, the decrease in NADPH absorbance was noticed as NADPH was converted to NADP. Given that the GPx enzyme is the rate-limiting component of the linked processes, it was directly proportional to the activity in the samples.

6.3. Reduced glutathione (GSH) level:

GSH was estimated by the colorimetric method as described by (Bahrami *et al.*, 2016; Ellman, 1959) using the kit supplied by Biodiagnostic com., Egypt. The reduction of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) with glutathione (GSH) results in the production of a yellow chemical in this approach. The absorbance of the reduced chromogen at 400 nm was directly related to the GSH content.

6.4. Superoxide dismutase (SOD):

SOD kit was obtained from Biodiagnostic com., Egypt. SOD activity was detected (Colorimetric Method) (Nishikimi *et al.*, 1972), by evaluating the rise in 560 nm absorbance for 5 minutes after adding PMS to the reaction mixture in the sample.

7. Statistical analysis:

The statistical package tool for social science (SPSS) was used for all statistical analyses (IBM SPSS Statistics for Windows, Version 25.0, 2017). The data was presented using mean ± SEM. The independent sample T-test was used for statistical analysis. The significance level was chosen at ($P < 0.05$).

RESULTS

1. Effect of malathion administration on testosterone level in serum in rats

The result showed a significant ($P < 0.05$) decrease in the mean testosterone hormone levels in the malathion-treated group (2.00±0.03) in comparison to the control group (2.24±0.04).

2. Effect of malathion administration on sperm parameters in rats:

Data output is shown in table (1) and photomicrograph (1) indicated that the malathion-treated group has a highly significant ($P < 0.05$) decrease in sperm counts and viability, while the malathion group has a highly significant ($P < 0.05$) increase in sperm abnormalities.

3. Effect of malathion administration on oxidative stress biomarkers in rats:

Concerning the effect of malathion on oxidative stress biomarkers, data output in table (2) showed that testicular activities/levels of SOD, GPx, and GSH were significantly ($P < 0.05$) decreased in the malathion-treated group when compared to the control group. On the other hand, MDA levels were significantly ($P < 0.05$) increased in the malathion-treated group when compared to the control group.

Table 1: Effect of malathion administration on sperm parameters in rats:

	Sperm counts (%)	Sperm viability (%)	Sperm morphology (%)
Control	73.06±0.2	70.6±0.5	96.8±0.13
Malathion	66.1±0.5 *	53.5±0.6 *	95.0±0.14 *

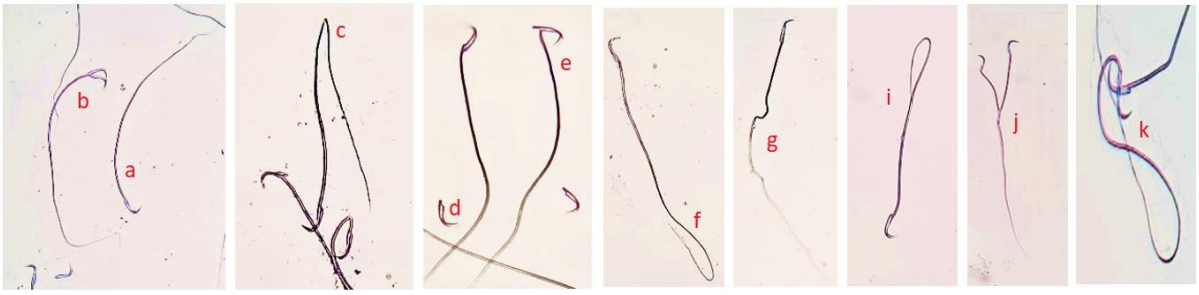
The data is presented as the mean ± SEM (n = 10). (*) star indicate statistical significance at P < 0.05.

Table 2: Effect of malathion administration on oxidative stress biomarkers (MDA, GPx, GSH and SOD) in rats:

	SOD (U /g. T)	MDA (nmol / g. T)	GPx (U/g. T)	GSH (mmol / g. T)
Control	512±1.5	21.3±0.05	7574.9±52.72	546.13±7
Malathion	422±2.7 *	34.5±0.04 *	6500.3±42.06 *	317.48±4.53 *

(SOD) superoxide dismutase, (MDA) Malonaldehyde, (GPx) glutathione peroxidase, (GSH) glutathione. The data is presented as the mean ± SEM (n = 10). (*) star indicate statistical significance at P < 0.05.

Photomicrograph (1):



The photomicrograph shown Alkaline methyl-violet stained semen film of malathion and treated group rats showed few abnormal sperms morphology (a) normal sperm, (b) double head, (c) pin tail, (d) broken head, (e) detached head, (f) hooked tail, (g) corrugated sperm, (i) folded tail, (j) biforked sperm, (k) twisted sperm.

DISCUSSION

Organophosphorus pesticides (OPPs) are one of the most extensively used types of pesticides in Egypt (el Okle *et al.*, 2022). Exposure is a major public health problem, which plays an essential part in the production process due to its effects on pathogen infection prevention and yield increase. However, throughout the early stages of OPP development and application, toxicological consequences and the issue of environmental pollution were concerns (Fu *et al.*, 2022). Acute and sub-chronic exposure to OP also induces oxidative stress by generating free radicals and producing lipid peroxidation (Eken, 2021). During the metabolism of pesticides compound in inebriated rats, superoxide anions, hydroxyl radicals, and H2O2 were generated (Yousef *et al.*, 2006). The metabolism and detoxification of ROS are facilitated by the antioxidants glutathione and enzymes associated with glutathion (Knapen *et al.*, 1999) e. The "redox-cycling" activity of OPs, in which They easily take an electron to create free radicals, and then they transmit those free radicals to oxygen to create superoxide anions, which then dismutated to produce hydrogen peroxide, may be the basis of their toxicity in the production of oxidative stress, or through ROS generation by alterations in normal antioxidant homeostasis which leading to antioxidant depletion, If the

demand for continuous antioxidants is not maintained (Banerjee *et al.,* 1999; Kovacic, 2003; Vidyasagar *et al.,* 2004).

Malathion is an organophosphorus (OP) insecticide that has been shown to disrupt sperm quality and impair reproductive hormone synthesis by inducing a cascade of oxidative stress (Omar *et al.,* 2022b). (Massoud, 2022) suggested that malathion affects the germinal and somatic cells of the testis and lowers testosterone activity, which inhibits spermatogenesis by causing Sertoli cells and germ cells to become damaged and vacuolated.

Various study investigations have shown that acute toxicity of malathion induces oxidative damage as well as elevations in MDA levels, while depleting the GSH, GPx activity, and reduces the activity of antioxidant enzymes such as SOD (Akbel *et al.,* 2018; Morsi *et al.,* 2022), The results of our current investigation demonstrated that long time malathion administrated even low dose 1/50 of LD50 caused a decrease in testosterone level and increase oxidative stress, which was confirmed by a rise in serum MDA levels and a fall in GPx, GSH, and SOD levels. Furthermore, (Akbel *et al.,* 2018) reported that malathion at a dose of (100 mg kg–1 day–1 by the oral route daily for 28 days in male rats) increases oxidative stress and decreases the antioxidant status. Because MDA is a persistent end product of lipid peroxidation, it has the potential to be used as an indirect measure of cumulative lipid peroxidation. (Hozyen *et al.,* 2020). Our findings show a direct correlation between the degrees of lipid peroxidation in the tissues of malathion-intoxicated rats and the levels of GSH and GPx in those tissues. that had the highest levels of lipid peroxidation and the lowest concentrations of both GSH and GPx.

(Omar *et al.,* 2022b) have reported that malathion exposure significantly reduced sperm count and motility as well as serum testosterone levels when using three different concentrations (25 mg/kg, 50 mg/kg, and 100 mg/kg) when using it for two and four weeks, which is consistent with the findings of our study at a dose of 27 mg/kg (3 times/week) for 60 days of exposure to malathion.

By studying the sperm parameters, similar results were declared by (Abo El-Atta & Ahmed, 2020; Moridi *et al.,* 2018; Uzun *et al.,* 2009), In their investigation into the toxic effects of malathion on testicular tissue, they reported significant reductions in sperm counts and motility, as well as an increase in abnormal sperm morphology. Although they used different concentrations and durations. This demonstrates that even at low concentrations, exposure to malathion residues in food or water can have substantial long-term harmful impacts on human and animal health.

CONCLUSIONS

From the aforementioned information, it could be concluded that malathion is a reproductively hazardous chemical pesticide that has a major negative impact on testosterone levels in serum and oxidative status in the testes of male rats. Moreover, Long term with time interval administration of malathion was associated with a significant reduction in sperm count and viability and increased abnormal sperm morphology.

Availability of data and materials

Please contact the author for data requests.

Authors' contributions

All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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التأثير طويل المدى لتعاطي الملاثيون على هرمون التستوستيرون ، والإجهاد التأكسدي، وخصائص الحيوانات المنوية في الفئران

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مبيدات الآفات العضوية الفسفورية هي واحدة من أكثر أشكال مبيدات الآفات استخدامًا في مصر حتي الآن والتي يمكن أن تؤثر سلبًا على الخصوبة في كل من الحيوانات والبشر. يهدف البحث الحالي إلى دراسة التأثيرات المحتملة لتعاطي الملاثيون لفترات طويلة على هرمون التستوستيرون في الدم، والمؤشرات الحيوية للتوتر التأكسدي في أنسجة الخصية وخصائص الحيوانات المنوية للفئران كنموذج تجريبي. تم تقسيم عشرين فأر ذكور من نوع ويستار إلى مجموعتين: المجموعة الضابطة (ن = ١٠) والمجموعة المعالجة بالملاثيون (ن = ١٠) بناءً على الدواء الذي تم تناوله عن طريق الفم بالتزقيم (٣ مرات / أسبوع لمدة ٦٠ يومًا). أثر إعطاء الملاثيون لفترة طويلة سلبًا على مستوى هرمون التستوستيرون، وعدد الحيوانات المنوية، وحيويتها، وأشكالها عند مقارنتها بالمجموعة الضابطة. زاد بيروكسيد الدهون (MDA) بشكل ملحوظ في المجموعة المعالجة بالملاثيون بالمقارنة مع المجموعة الضابطة. من ناحية أخرى، تسبب تناول الملاثيون في انخفاض كبير في أنشطة الجلوتاثيون بيروكسيديز (GPx)، وانخفاض الجلوتاثيون (GSH)، وإنزيمات سوبر أكسيد ديسموتاز (SOD) في أنسجة الخصية في ذكور الجرذان. في الختام، فإن تناول الملاثيون على المدى الطويل كان له آثار ضارة على مستوى هرمون التستوستيرون وحالة أكسدة الخصية وكذلك جودة السائل المنوي في ذكور الجرذان. بناء على ذلك فإنه لا بد من الاستقصاء عن بقايا مبيدات الفوسفور العضوي في النباتات لحماية صحة المستهلك.