International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:8, No:8, 2014

Protective Effect of *Melissa officinalis* L. against Malathion Toxicity and Reproductive Impairment in Male Rats

M. M. Seif, F. A. Khalil, A. A. K. Abou Arab, A. S. Abdel- Aziz, M. A. Abou Donia, Sh. R. Mohamed

Abstract**—**Malathion (ML) is a well known pesticide commonly used in many agricultural and non-agricultural processes. Its toxicity has been attributed primarily to the accumulation of acetylcholine (Ach) at nerve junctions, due to the inhibition of acetylcholinesterase (AChE). The aim of the current research was to study the protective effect of the melissa plant extract against reproductive impairment induced by malathion in 32 male albino rats, and the biological experiment was divided into four groups (8 in each) that given malathion (27 mg/kg; 1/50 of the LD_{50} for an oral dose) and/or *Melissa officinalis* (MO) extract (200mg/kg/day) by gavages technique. The sperm counts, sperm motility, sperm morphology, FSH, LH, and testosterone levels had been determined in testes homogenate at the end of the experiment. It is worthy to report that, rats treated with melissa extract did not show a significant difference when compared with the control group, while rats given malathion alone had significantly lower sperm count, sperm motility, and significantly higher abnormal sperm numbers, than the untreated control rats as well as having significantly lower serum FSH, LH, and testosterone levels compared with the control group. Administrations of melissa extract restore all mentioned histological parameters towards the control group and the melissa extract had a strong positive protective effect against malathion toxicity. Results the of biological parameters were confirmed by the histological examination of rat testes and indicated that, both control and melissa groups showing normal seminiferous tubules, while malathion group testicular tissues had necrosis, edema in the seminiferous tubules and degeneration of spermatogonial cells lining the seminiferous tubules with incomplete spermatogenesis. The use of melissa against malathion improved the histological picture and showing normal seminiferous tubules with complete spermatogenesis and almost there was no histopathological changes could be noted.

Keywords**—**Malathion, *Melissa officinalis* L., Reproductive toxicity, Rats.

I. Introduction

ORGANOPHOSPHATE (OP) pesticides are widely used for agriculture, vector control and domestic purposes. About 70% of the pesticides in current use are OP compounds which constitute a total consumption of around 90 million pounds per year. However the uncontrolled application of these pesticides in agriculture and public health operations has increased the scope of ecological imbalance and thus many non-target organisms have become a victim [1].

Pesticides are also frequently employed in medicine and industry. Residual amounts of organophosphate (OP) pesticides have been detected in the soil, water bodies, vegetables, grains, and other food products [2].

Modern agricultural practices depend on the production and use of pesticides to control pests and increase productivity. There are presently over 1000 chemicals that are classified as pesticides [3].

Malathion [O, O-dimethyl-S-(1, 2-dicarbethoxyethyl) phosphorodithioate] is an OP pesticide that is widely used in agricultural and household applications to control pests. Malathion is also extensively used for mosquito eradication and as an animal ectoparasiticide and human miticide. The widespread use of malathion and the high rates of food contamination could lead to humans, animals, and birds being exposed to high levels of this pesticide chemical [4].

According to the Environmental Protection Agency Malathion is one of the most commonly used insecticides in the United States, with an estimated use of 30 million pounds a year. Approximately 60% of this total is used for various insect eradication programs. [5].

Malathion is known to inhibit acetylcholinesterase activity in target tissues [6] and has been found to affect the mammalian reproductive system. For example, mice treated with intraperitoneally-injected malathion showed significantly higher frequencies of abnormal sperm [7].

Moreover, malathion exposure has been shown to significantly decrease the sperm count of mice [8]. In the same concern, in Egypt when mice fed stored wheat that had been treated with a commercial malathion insecticide developed two kinds of genetic damage and increased of abnormalities in spermatocytes [9].

Many insecticides are hydrophobic molecules that bind extensively to biological membranes, especially phospholipid bilayers [10], and they may damage the membranes by inducing lipid peroxidation.

The plant extracts have an important role as antioxidant agents against the hazard of food contaminants for example, there are many reports indicated that a low polar extract of lemon balm leaves, especially its essential oil, had good antioxidant and anti-tumoral activities [11].

M. M. Seif is with the Food Toxicology and Contaminants Department, National Research Center, Dokki, Cairo, Egypt (phone: +201281052247; fax: +233370931; e-mail: seif.eg@gmail.com).

A.A.K. Abou Arab, M. A. Abou Donia, and Sh. R. Mohamed are with the Food Toxicology and Contaminants Department, National Research Center, Dokki, Cairo, Egypt (e-mail: aak abouarab2007@yahoo.com, maboudonia1@yahoo.com, sheriframzy4@gmail.com).

F. A. Khalil and A. S. Abdel- Aziz are with Department of Animal Physiology, Fac. of Agriculture, Ain-Shams University, Cairo, Egypt (e- mail: faroukabdalla2008@yahoo.com, abdelaziz.ahmed75@yahoo.com).

Lemon balm (*Melissa officinalis* L.) is an herb, member of the Lamiaceae family and it is used as a culinary flavoring often in combination with other herbs such as spearmint. In medicinal field lemon balm has many uses such as mild sedative or calming agent, it can improve mental performance, it is claimed to have antiviral and antibacterial properties against herpes simplex [12] and that lemon balm extract has an exceptionally high antioxidant activity and flavonoids and can provide antioxidant activity [13]. Other phytochemicals, which can have an antioxidant effect of lemon balm, are: rosmarinic acid, phenolic acids, caffeic acids and terpenes. It has been reported that lemon balm possesses strong antioxidant activity and lemon balm extract can improve the oxidative stability of sunflower oil [14]. The aim of the current research was to study the protective effect of the Melissa plant extract against reproductive impairment induced by malathion in male rats.

II. Materials and Methods

A. Materials

1. Animals

Thirty two male Sprage Dawley albino rats of three months of age and 135-140g of body weighing were obtained from an animal house colony, National Research Center, Giza, Egypt, all animals were maintained on a standard lab diet (protein: 160.4; fat: 36.3; fiber: 41 g/kg and metabolic energy 12.08 MJ), and housed in a room free from any source of chemical contamination, artificially illuminated and thermally controlled.

2. Chemicals

Malathion was obtained from agriculture ministry office. All solvents used in the study were high grade and were obtained from Sigma Chemical Co. (St. Luis, Mo, USA).

3. *Melissa officinalis* L. Plant

Arial parts of *M. officinalis* were obtained from the herbarium, National Research Center, Dokki, Cairo, EGYPT.

4. Kits

Kits of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), LH and FSH, and testosterone were obtained from Biodiagnostic Co., Dokki, Giza, EGYPT.

B. Methods

1. Preparation of *Melissa officinalis* Extract

The dried arial parts powder of *M. officinalis* L. (100 g) was soaked at room temperature in 1000 ml (70% ethanol) and extracted for 1 week. On the seventh day, the ethanolic extract was stirred for 12 h and filtered through a Whatman No. 1 filter paper, and then solvent was fully evaporated with a rotary evaporator (Panchun Scientific Co.) at 35ºC. The ethanolic extract was freeze dried for further use.

2. Experimental Animals and Treatment Schedule

After an acclimatization period of 1 week, animals were equally divided into four groups (8 rats in each) and housed in filter top polycarbonate cages. The different groups were treated as following**:**

a. Control Group

Corn oil at a dose of 2 ml per animal was given via gavage, once a day.

b. *Melissa officinalis* Extract-Treated Group

Melissa officinalis freeze dried was dissolved in corn oil (10 g/L), and the rats were given it at 200 mg/kg bw per day by gavage.

c. Malathion-Treated Group

The rats were given daily and orally malathion at a dose of 27 mg/kg bw (1/50 of the LD_{50}) in corn oil via gavage.

d. *Melissa officinalis* Extract Plus Malathion-Treated Group

The rats were given daily and orally *Melissa officinalis* extract dissolved in corn oil (200 mg/kg bw) by gavage, and 30 min later, malathion dissolved in corn oil (27 mg/kg bw per day) was administered via gavage.

At the end of the experimental period (12 weeks) of treatment, the eight rats in each group were sacrificed and dissected, and tissue samples were taken to determine testicular sperm counts, epididymal sperm motility, epididymal sperm morphology and antioxidant parameter were investigated in testes homogenate. Testes samples were also subjected to light microscope. Blood samples were collected for assaying the levels of serum hormones, including luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone.

C. Testicular Sperm Count

One testis of each rat was placed in 1 ml of phosphate buffer saline immediately after dissection. The tunica albuginea was cut by surgical blades and removed, and the remaining seminiferous tubules were mechanically minced by using surgical blades in 1 ml of phosphate buffer saline. Residual cauda epididymidis were minced with scissors and filtered through gauze. Filtered samples were diluted with saline containing 0.5% formalin. This solution was infused into a Neubauer-type hemocytometer (Erma, Japan) for microscopic observation. The data were expressed as the total number of sperm per cauda epididymal tissue weight. Every sample was evaluated without information about its treatment. [15].

D. Sperm Motility Analysis

The sperms were collected as quickly as possible after a rat was sacrificed. The cauda epididymis was placed in 1 ml of 37ºC phosphate buffer saline solution and cut by surgical blades into approximately 1 mm3 pieces. The solution was pipetted several times to homogenize the sperm suspension. One drop of the suspension was placed on a slide, covered by a 24 x 24 mm cover slip, and evaluated under a phase contrast microscope at 200x magnification. The sperm was categorized on the basis of their motility as motile or immotile. The results were recorded as the percentage of sperm motility [16].

E. Epididymal Sperm Morphology

The sperm solution was smeared on glass slides, fixed in methanol and dried. The slides were stained later according to Bryan's method [17]. A total of 200 intact sperm were examined for morphological abnormality under the microscope. Abnormal heads were classified as straight, banana-shaped, and other unclassified abnormalities according to the method [18].

F. Hormone Assays

The LH and FSH levels in the blood serum were measured by using an automated immunofluorescent assay-based commercial kits and a Brahms Kryptor immunoassay analyzer (Brahms LH Kryptor 820.050 and Brahms FSH Kryptor 818.050, respectively). The testosterone levels were measured by using a chemiluminescence immunoassay- based commercial kit (Access testosterone 33,560) and an Access immunoassay analyzer (Beckman Coulter).

G. Histopathological Study

For histological examination, testis tissues were dissected using paraffin wax ordinary microtechnique. Paraffin sections were cut into 6 µm-thick slices and stained with hematoxylin and eosin for light microscopic examination. The sections were viewed and photographed using an Olympus light microscope (Olympus BX51, Tokyo, Japan) with an attached camera (Olympus E-330, Olympus Optical Co., Ltd., Japan. Two slides were prepared from each testicular tissue.

H. Statistical Analysis

All data were statistically analyzed using the General Linear Models Procedure of the Statistical Analysis System (SAS, 1982). The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio [23]. All statements of significance were based on probability of $P < 0.05$.

III. Results

A. Evaluation of Testicular Sperm Counts

The results showed that the *Melissa officinalis* extract-treated group and *Melissa officinalis* extract plus malathion-treated group did not differ significantly from the control group at the end of the experimental period in terms of sperm counts, but the malathion-treated rats had significantly lower sperm counts than the control group ($P < 0.05$; Table I). It is worthy to report that melissa extract can protect the sperm against malathion stress and maintains its efficiency as a control group.

B. Evaluation of Sperm Motility

The effect of malathion and *Melissa officinalis* extract as an antioxidant agent was evaluated on the sperm motility and noted that the control and melissa rat groups were in normal case and the sperm motility was in normal form for both groups, while malathion- treated rats had significantly lower sperm motility than the control rats. *Melissa officinalis* L. extract rats did not differ significantly from the control rats in terms of their total epididymal sperm motility at the end of the experimental period. ($P < 0.05$; Table I). However, the rats treated with *Melissa officinalis* L. extract plus malathion showed significant improvement in sperm motility when compare with malathion- treated rats.

C. Evaluation of Epididymal Sperm Morphology

The epididymal sperm morphology was measured in tests of rat groups treated with malathion or malathion plus melissa extract. At the end of the experiment, the *M. officinalis* L. extract-treated group and control rats did not differ significantly in terms of their rates of abnormal sperm morphology, but the malathion-treated rats had significantly higher abnormal sperm morphology rates than the control rats ($P < 0.05$; Table I). It is worthy to report that the *M. officinalis L.* extract plus malathion-treated rats had significantly improved the abnormal sperm morphology of rats when compared with animal treated with malathion alone ($P < 0.05$; Table I).

TABLE I
CHRONIC EFFECT OF MALATHION ON THE SPERM MOTILITY, SPERM MORPHOLOGY, AND ABNORMAL SPERM MORPHOLOGY IN RATS

Groups	Sperm count (million/ml)	Sperm motility (%)	Abnormal sperm morphology (%)
Control	30.38 ± 0.84^a	74.05 ± 0.55^a	1.75 ± 0.06^a
MO	28.08 ± 0.19^a	72.73 ± 0.34^a	1.81 ± 0.03^a
ML	14.64 ± 0.45^b	22.26 ± 0.51^b	3.94 ± 0.28^b
MO + ML	29.18 ± 0.45^a	72.35 ± 0.46^a	1.82 ± 0.07^a

Within each column, means superscripted with different letters are significantly different ($P \leq 0.05$); control= untreated group, MO= *Melissa officinalis*; ML= malathion; MO+ ML= *Melissa officinalis* + malathion

D. Serum Levels of FSH, LH, and Testosterone

The control and *M. officinalis* L. extract-treated rats had almost similar values of FSH, LH as well as testosterone levels (Table II and Figs. 1, 2), but the malathion-treated rats had significantly lower plasma FSH, LH and testosterone levels than the control rats. While, the group treated with *M. officinalis* L. extract plus malathion showed significant improvement in terms of plasma FSH, LH and testosterone levels when compared with the group of malathion alone (Table III and Figs. 3, 4).

E. Histopathological Findings in the Testis

The biochemical parameters of rat groups were confirmed by the histological study which showed that the spermatogenic cells and sertoli cells in the seminiferous tubules of the MO-treated group and control rats were normal in structure (Figs. 3 A and B).

TABLE II
CHRONIC EFFECT OF MALATHION ON SERUM LEVELS OF FSH, LH AND TESTOSTERONE IN RATS

Groups	FSH (lU/ml)	LH (lU/ml)	Testosterone (ng/ml)
Control	4.13 ± 0.01^a	1.22 ± 0.01^a	3.64 ± 0.2^a
MO	4.10 ± 0.01^a	1.21 ± 0.01^a	3.61 ± 0.2^a
ML	2.83 ± 0.04^b	0.69 ± 0.02^b	2.11 ± 0.12^b
MO+ ML	4.06 ± 0.02^a	1.20 ± 0.01^a	3.55 ± 0.3^a

Within each column, means superscript with different letters are significantly different ($P \leq 0.05$); Follicle Stimulating Hormone (FSH); Luteinizing Hormone (LH); control= untreated group, MO= *Melissa officinalis*; ML= malathion; MO+ ML= *Melissa officinalis* + malathion.

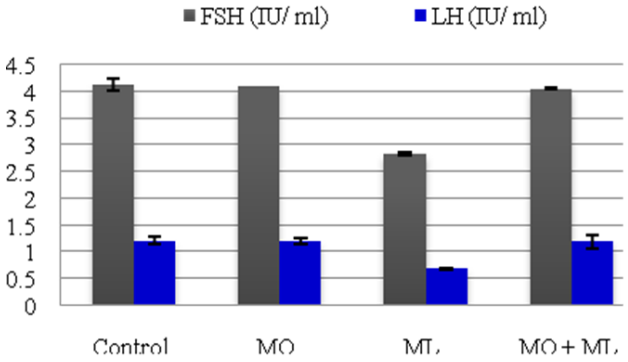

Fig. 1 Chronic effect of malathion on serum level of FSH and LH (IU/ml) in rats

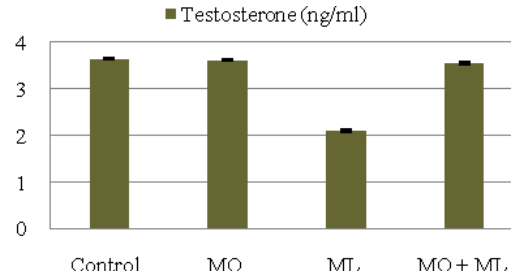

Fig. 2 Chronic effect of Malathion on serum levels of testosterone (ng/ml) in rats

Leydig cells were found in the interstitial connective tissue between the seminiferous tubules, and the tubules appeared to be uniform in size and shape. They were lined by regularly arranged rows of spermatogenic cells at different stages of maturation (Fig. 3 A). In contrast, after 12 weeks of malathion exposure, there were fewer spermatogenic cells in some of the seminiferous tubules, necrosis in some seminiferous tubules and edema in interstitial tissue compared with control groups (Figs. 3 A and C).

Moreover, after 12 weeks of MO extract plus malathion treatment, there were normal cells and complete spermatogenesis and no showing any histopathological changes in the seminiferous tubules (Fig. 3 D).

IV. Discussion

Malathion is a widely used pesticide that affects a variety of organs. Epidemiological research into the acute and chronic toxicity of Malathion indicates that this chemical is highly toxic to mammals [24]. Mammals are expected to be adversely affected by oral, dermal and inhalation exposure to Malathion [25]. Malathion not only has toxic effects on mammals, but also has toxic effects on fish, chicks and non-target invertebrates [26]. The oral LD_{50} of malathion for male rats is 1350 mg/kg [27]. However, when [6] gave rats 100 mg/kg malathion intragastrically, they observed hepatic damage and biochemical changes. In the present study, even though malathion was given at 1/50 of the oral LD_{50}, we observed pathological changes in rat testes, although none of the rats died during the experimental period.

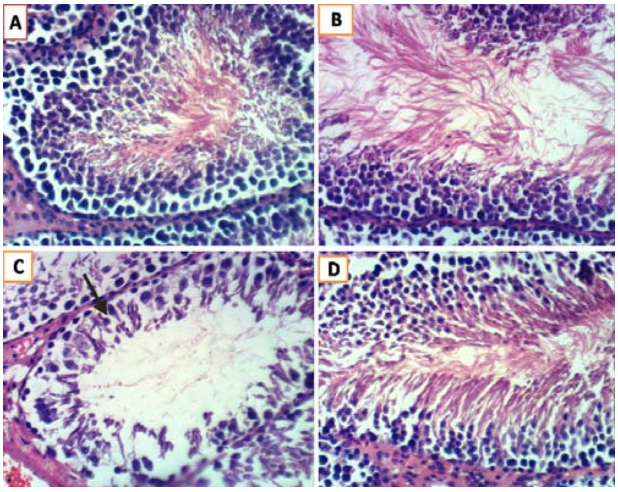

Fig. 3 (A) Testicular section of control group showing normal seminiferous tubules with complete spermatogenesis; (B) MO-treated group, showing normal seminiferous tubules with complete spermatogenesis; (C) ML-treated group, Showing Necrosis (↘) in the seminiferous tubules and showing degeneration (↘) of spermatogoneal cells lining the seminiferous tubules with incomplete spermatogenesis; (D) ML plus MO- treated group, showing no histopathological changes and improved histological picture, (H & E X 400)

It has been shown that pesticides can cause various histopathological and cytopathological changes in the reproductive system of male mammals [28]. These changes include decreased spermatogenesis and sperm counts. For example, acephate, an OP insecticide, decreases the number of spermatogenic cells in the testes [29], while [30] reported that phosphorothionate, an OP insecticide, inhibits spermatogenesis.

In addition, [31] found that male rats exposed to phoxim, an OP insecticide, along with fenvalerate, a pyrethroid insecticide, show decreased daily sperm production. In addition, OP insecticides not only decrease sperm counts, but also reduce sperm motility [30].

Moreover, [28] demonstrated that pesticide-exposed experimental animals produce significantly higher numbers of dead or abnormal sperm. In another studies, they found that rats exposed for 4 and 7 weeks to a close relative of malathion, methyl parathion, had decreased sperm counts. Malathion and methyl parathion have also been reported to reduce sperm motility and viability, and to increase abnormal sperm count, these changes are concentration-dependent and intensify the longer the animals are exposed. Malathion exposure also reduces sperm counts has been shown previously by [7], who found that mice treated intraperitoneally for 18 days with malathion exhibited decreased sperm counts.

In Egypt, mice fed with stored wheat that had been treated with a commercial malathion insecticide developed two kinds of genetic damage and increased of abnormalities in spermatocytes [9].

Similarly, these rats exhibited a significant decrease in sperm motility and increased abnormal morphology rates. It is

likely that these effects of malathion and other OPs relate, at least in part, to their ability to cross the blood–testis barrier [28], after which they induce oxidative stress and lipid peroxidation that damages the biological membranes in the testes. This in turn may cause the degeneration of the spermatogenic and Leydig cells, which disrupts spermatogenesis and reduces sperm counts. Supporting this is the fact that we found that subacute exposure to malathion-induced histopathological changes in the seminiferous tubules, namely, necrosis and edema in the seminiferous tubules and interstitial tissue.

The sperm themselves may also be damaged by the oxidative effects of OPs, which affect the activities of mitochondrial enzymes and the structure of the microtubulus in the sperm. This in turn reduces their motility. That reactive oxygen species may contribute to infertility caused by defective sperm function has been reported previously [32]. Another way OPs affect male reproductive function is to damage DNA [33]. Increases in abnormal sperm counts and the disruption of spermatogenesis are important indicators of genetic damage in pesticide-exposed mammals [34]. Since sperm morphology is controlled by various autosomal and Y-specific genes [35], DNA damage may also reduce sperm motility. Supporting the notion that OPs like malathion exert their deleterious effects by promoting destructive oxidation of lipids, proteins and DNA within the testis is our finding that co-treatment of malathion-exposed rats with *M. officinalis* L. ameliorated the effects of malathion on sperm counts, motility and morphology, and the integrity of the testis.

In our research the role of *Melissa officinalis* extract against the health hazard of malathion in rats was studied. Balm *(Melissa officinalis* L.) is very useful for nervous agitation, and for promoting sleep, and ameliorates functional gastrointestinal complaints [36].

In folk medicine, balm is recommended as a plant juice, cream or tea infusion for nervous complaints, lower abdominal disorders, gastric complaints, hysteria and melancholia, chronic bronchial catarrh, migraine, nervous debility, toothache, earache, headache and high blood pressure and, externally, for rheumatism, nerve pains and stiff necks (compress) [37]. Balm contains volatile oil, glycosides of the alcoholic or phenolic components of the volatile oil (eugenol glucoside) [38], caffeic acid derivatives (rosmarinic acid), flavonoids (cynaroside, cosmosin, rhamnocitrin, isoquercitrin), phenolic acid (carnosic acid), and triterpene acids (ursolic and oleanolic acid). The last two compounds are well known antioxidants [39].

It is essential oil is considered to be the therapeutic principle mainly responsible for most of the activities mentioned, but plant phenolics, especially rosmarinic acid, are also considered as an important factor in *M. officinalis* L. therapeutic effects and extract may have the potential to prevent oxidative damage in vivo by preventing free-radical-mediated oxidative stress [13].

Its potentially active components primarily include monoterpenoids and sesquiterpenes, including geranial, neral, 6-methyl-5-hepten-2-one, citronellal, geranyl-acetate, β-caryophyllene and β-caryophyllene-oxide, and1, 8 cineole [40].

Recently, [41] have demonstrated that *M. officinalis* exhibits antigenotoxic/ antimutagenic properties in mice. So in our work the effect of melissa extract as a main source of antioxidants against malathion hazard in rat testes was studied and found that the *M. officinalis* extract has antioxidant activity agent and could improve the biochemical and histochemical picture of rat testes against toxicity of malathion or alone.

Organphosphorus may also affect male reproductive function by decreasing FSH, LH and testosterone levels. Significant alterations in FSH, LH and testosterone levels have been reported after exposure to certain pesticides [42]. For example, exposure to methyl parathion has been reported to significantly reduce LH and testosterone levels. However, other researchers have found that pesticides elevate FSH and LH levels [43].

LH and FSH activity depends on both the quantity of these hormones and the number of specific receptors in the testis. It has been shown that exposure to environmental contaminants adversely affects testicular function by decreasing pituitary LH secretion and reducing Leydig cell steroid genesis [44]. In our study, the FSH and LH levels in the malathion-treated rats were significantly lower than the levels in the control rats at the end of the 12 weeks. Thus, subacute malathion exposure suppressed FSH and LH secretion. These results may be explained by the putative androgen receptor antagonistic property of malathion it is known that androgen receptor antagonist substances can alter the glycosylation of gonadotrophins, which results in the suppression of FSH and LH levels [45]. Together with gonadotrophins, testosterone is a key hormone that regulates spermatogenesis. The secretion of testosterone by the Leydig cells is dependent upon the secretion of LH by the pituitary gland. Various OPs have been studied for their effect on testosterone levels.

In this study, malathion exposure for 12 weeks was associated with a decrease in serum testosterone levels. This was reflected on same pathological changes in the seminiferous tubules and spermatogenesis processes. Thus, in summary, we found that a low dose of malathion causes testicular toxicity, and the Melissa extract posses the protective role against toxicity of malathion on rat testes since it contains the antioxidant components and could improve the sperm counts, motility and morphology. And also protect malathion-exposed rats from the effects of malathion on FSH, LH and testosterone levels.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Prof. Khayria M. Naguib. Head of Mycotoxins Central Lab., National Research Center, Cairo, Egypt for her great supports for this work.

REFERENCES

[1] B. K. Das, and S. C. Mukherjee. Chronic toxic effects of quinalphos on some biochemical parameters in *Labeo rohita* (Ham). Toxicol. Lett. vol. 114, pp 11–18. 2000.
[2] T. S. Poet, A. A. Kousba, S.L. Dennison and C. Timchalk. Physiologically based pharmacokinetic/pharmodynamic model for the organophosporus pesticide diazinon. Neurotoxicology, vol., 25 pp.1013–1030.2004.
[3] D. Zeljezic and V. Garaj-Vrhovac. Sister chromatid exchange and proliferative rate index in the longitudinal risk assessment of occupational exposure to pesticides, Chemosphere vol., 46, pp. 295–303.2002.
[4] N. Suresh, J. K. Babu, G. S. Malik, M. Aggarwal, and V. Ranganathan. (2006). Effects of subchronic Malathion exposure on the pharmacokinetic disposition of pefloxacin. Environ. Toxicol. Pharmacol. Vol.,22.pp.167–171.
[5] C. Cox. Malathion, J. Pestic.vol., 4.pp.10–15.2003.
[6] R. Rezg, B. Mornagui, S. El-Fazaa and N. Gharbi. Caffeic acid attenuates malathion induced metabolic disruption in rat liver, involvement of acetylcholinesterase activity. Toxicology vol., 250.pp. 27–31.2008.
[7] H. R. Contreras and E. Bustos-Obregón. Morphological alterations in mouse testis by a single dose of malathion. J. Exp. Zool. Vol.,284. Pp.355–359. 1999.
[8] P. Gonzáles-Hormazabal and E. Bustos-Obregón. Effect of a single dose of malathion on spermatogenesis in mice. Asian J. Androl. Vol.,5.pp. 105–107.2003.
[9] S. A. Amer, M.A. Fahmy, F. A. Aly, and A. A. Farghaly. Cytogenetic studies on the effect of feeding mice stored wheat grain treated with malathion. Mutat. Res. Vol., 15.pp. 1-10.2002.
[10] A. G. Lee, J. M. East, and P. Balgauvy. Interactions of insecticides with biological membranes. Pestic. Sci. vol.,32pp.317–327.1991.
[11] A. C. De Sousa, D. S. Alviano, A. F. Blank, Alves, P. B. C. S. Alviano and C. R. Gattass. *Melissa officinalis L* essential oil: antitumoral and antioxidant activities. J. Pharm Pharmacol vol., 56.pp.677–68.2004.
[12] P. Schnitzler, A. Schuhmacher, and A. Astani. *Melissa officinalis* oil affects infectivity of enveloped herpesviruses". Reichling, J., vol.,15.pp.734-740.2008.
[13] K. Dastmalchi, H. D. Dorman, P. P. Oinonen, Y. Darwis, I. Laakso and R. Hiltunen. Chemical composition and in vitro antioxidative activity of a lemon balm (*Melissa officinalis L.*) extract. Food Sci. Technol. Vol.,41.pp.391–400.2008.
[14] J. Hohmann, I. Zupko, D. Redei, M. Csanyi, G. Falkay and I. Mathe (1999). Protective effects of the aerial parts of *Salvia officinalis, Melissa officinalis* and *Lavandula angustifolia* and their constituents against enzyme-dependent and enzyme-independent lipid peroxidation, Planta Medica, vol., 65.pp.576-584.1999.
[15] A. Okamura, M. Kamijima, E. Shibata, K. Ohtani, K. Takagi, J. Ueyama, Y. Watanabe, M. Omura, H. Wang, G. Ichihara, T. Kondo, and T. Nakajima. A comprehensive evaluation of the testicular toxicity of dichlorvos in Wistar rats. Toxicology vol., 213.pp. 129–137.2005.
[16] G. U. Fatma, K. Suna, D. Dilek, D. Filiz, and K. Yusuf. Malathion-induced testicular toxicity in male rats and the protective effect of vitamins C and E. journal of Food and Chemical Toxicology, vol., pp.47: 1903–1908. 2009.
[17] J. H. Bryan. An eosin-fast green-naphthol yellow mixture for differential staining of cytologic components in mammalian spermatozoa. Stain. Technol. Vol.,45.pp. 231–236.1970.
[18] K. Mori, M. Kaido, K. Fujishiro, N. Inoue, O. Koide, H. Hori, and I. Tanaka. Dose dependent effects of inhaled ethylene oxide on spermatogenesis in rats. Br. J. Ind. Med. Vol., 48.pp.270–274.1991.
[19] O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall. (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry vol., 19, 265.1951.
[20] H. H. Draper and M. Hadley. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol. Vol.,186.pp.421–431.1990.
[21] S. Marklund, and G. Marklund (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. European Journal of Biochemistry.47
[22] H. Aebi. Ctalase in vitro. Methods in Enzymology vol.,105.pp.121–126.1984.
[23] R. A. Waller and D. B.Duncan (1969). A Bayes rule for the symmetric multiple comparison problems. J. Am. Stat. Assoc. vol.,64.pp.1484-1503.
[24] M. Abdollahi, N. Jalali, O. Sabzevari, S. Nikfar and M. Fallahpour. Pesticide poisoning during an 18-month period (1995–1997) in Tehran. Iran. Iran. J. Med. Sci. vol.,24.pp.77–81.1999.
[25] J. W. Edwards, S. Lee, L. M. Heath, and D. L. Pisaniello (2007). Worker exposure and a risk assessment of malathion and fenthion used in the control of Mediterranean fruit fly in South Australia. Environ. Res. Vol.,103pp:38–45.2007.
[26] S. Sodhi, A. Sharma, A. S. Brar, and R. S. Brar. Effect of a tocopherol and selenium on antioxidant status, lipid-peroxidation and hepatopathy induced by malathion in chicks. Pestic. Biochem. Phys. Vol.,90.pp.82–86.2008.
[27] S. John, M. Kale, N. Rathore, and D. Bhatnagar. Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. J. Nutr. Biochem. Vol.,12.pp.500–504.2001.
[28] M. Uzunhisarcikli, Y. Kalender, K. Dirican, S. Kalender, A. Ogutcu, and F. Buyukkomurcu. Acute, subacute and subchronic administration of methyl parathion induced testicular damage in male rats and protective role of vitamins C and E. Pestic. Biochem. Phys. Vol.,87.pp.115–122.2007.
[29] A. T. Farag, M. H. Ewediah, and A. M. El-Okazy. Reproductive toxicology of acephate in male mice. Reprod. Toxicol. Vol.,14.pp.457–462.2000.
[30] I. A. Khan, B. V. Reddy, M. Mahboob, M. F. Rahman, and K. Jamil. Effects of phosphorothionate on the reproductive system of male rats. J. Environ. Sci. Health vol.,36.pp.445–456.2001.
[31] L. Xu, N. Zhan, R. Liu, L. Song, X. Wang. Joint action of phoxim and fenvalerate on reproduction in male rats. Asian J. Androl. Vol.,6.pp.337–341.2004.
[32] C. Latchoumycandane, K. C. Chitra, and P. P. Mathur. The effect of methoxychlor on the epididymal antioxidant system of adult rats. Reprod. Toxicol. Vol.,16.pp.161–172.2002.
[33] L. Sarabia, I. Maurer, and E. Bustos-Obregón. Melatonin prevents damage elicited by the organophosphorous pesticide diazinon on mouse sperm DNA. Exotoxicol. Environ. Saf. Vol.,72.pp.663–668.2009.
[34] V. R. Burruel, O. G. Raabe, J. W. Overstreet, B. M. Wilson and L. M. Wiley. Paternal effects from methamidophos administration in mice. Toxicol. Appl. Pharm. Vol.,165.pp.148–157.2000.
[35] J. Forejt. Spermatogenic failure of translocation heterozygotes affected by H- inbreed gene in mouse. Nature vol.,260.pp.143–145.1976.
[36] G. Kumel, L. Stoll and M. Brendel. (1991). Therapie mit rezeptfreien Topika. Deutsche Apotheker Zeitung, vol.,131.pp.1609.
[37] R. A. Cohen, L. S Kucera,. and E. Herrmann. Antiviral activity of *Melissa officinalis* (Limon Balm) extract. Proceedings of the Society for Experimental Biology and Medicine, vol.,117.pp.431–434.1964.
[38] E. Sarer, and G. Kokdil. Constituents of the essential oil from *Melissa officinalis*. Planta Medica, 57, 89.1991.
[39] W. Schultze, W. A. Kolnig, A. Hilker and R. Richter (1995). Melissenole. Deutsche Apotheker Zeitung, vol.,135.pp.557–577.
[40] G. Tittel, H. Wagner, R. Bos. Chemical-composition of the essential oil from melissa. Planta Med. Vol.,46.pp.91–98.1982.
[41] N. C. De Carvalho, M. J. F. Corrêa-Angeloni, D. D. Leffa, J. Moreira, V. Nicolau, P. A. Ama-ral, A. E. Rossatto and V. M. de Andrade. Evaluation of the genotoxicand antigenotoxic potential of *Melissa officinalis* in mice. Genet. Mol. Biol.vol.,34.pp.290–297. 2011.
[42] A. Elbetieha, and S. I. Daas. Assessment of antifertility activities of a bamectin pesticide in male rats. Ecotoxicol. Environ. Saf. Vol.,55.pp.307–313.2003.
[43] A. A. Mahgoub, and A. H. El-Medany. Evaluation of chronic exposure of the male rat reproductive system to the insecticide methomyl. Pharm. Res. Vol.,44.pp.73–80.2001.
[44] P. Murugesan, T. Muthusamy, K. lasubramanian, and J. Arunakaran. Effects of vitamins C and E on steroidogenic enzymes mRNA expression in polychlorinated biphenyl (Aroclor 1254) exposed adult rat Leydig cells. Toxicology vol.,22.pp.170–182.2007.
[45] R. Naz. Endocrine Disruptors, effects on Male and Female Reproductive Systems. CRC Press, Florida.1999