JOURNAL OF MARINE SCIENCES & ENVIRONMENTAL TECHNOLOGIES
Vol. 7, Issue No. 1 (June-2021)

ISSN (Print): 2413-5267
ISSN (Online): 2706-9966

Effect of The Recommended Dose of Dimethoate and Its Double on The Embryos and Testicular Tissues in Male Mice

Samira M. Sasi[1, *] and Nagia M. El-Ghoul[2]

[1)] Department of Zoology, Faculty of Science, University of Tripoli, Tripoli, Libya.
[2)] Biotechnology Center, Tripoli, Libya.

[*]**Corresponding author:** Samira_18_3@yahoo.com

تأثير الجرعة الموصى بها من مبيد الدايمثويت وضِعْفِها على الأجنة وأنسجة الخصية في ذكور الفئران

سميرة موسى ساسي[1،*] وناجية موسى الغول[2]

[1)] قسم علم الحيوان، كلية العلوم، جامعة طرابلس، طرابلس، ليبيا.
[2)] مركز بحوث التقنيات الحيوية، طرابلس، ليبيا.

Received: 07 March 2021; Revised: 27 June 2021; Accepted: 29 June 2021.

Abstract

Exposure to pesticides negatively have associated with many public health hazards infecting humans as infertility, therefore, the objective of this study was to investigate the effect of dimethoate on testicular cytoarchitecture of treated mice and on the embryos. Albino mice were divided into three groups of 9 each: the first group served as control and was given distilled water orally, whereas the second and third groups were given dimethoate at doses (0.1 and 0.2 mL dimethoate/100 mL distilled water) respectively for 20 days. At the end of the treatment, six mice from each group were weighed and sacrificed, the testis and epididymis weights were recorded, and histopathological lesions of tests were carried out. The obtained results revealed that dimethoate caused a decrease in the body and epididymis weights. Furthermore, dimethoate led to a significant decrease in the number of dead embryos and their weights. Histopathological examination demonstrated that this pesticide caused marked alterations in the microstructure of testicular tissues appeared as vacuoles, disorganization of the germinal epithelium, and degenerative changes in some seminiferous tubules as well as its effect on the embryos, therefore, more efforts should be conducted to protect our environment and health from these detrimental compounds and the search for safe methods for insect control.

Keywords: *Dimethoate, Fertility, Male mice, Testis.*

الملخص

ارتبط التعرض للمبيدات بحدوث مخاطر صحية عديدة تصيب الإنسان بما فيها نقص الخصوبة، لذلك كان الهدف من هذه الدراسة معرفة تأثير الدايمثويت على الأجنة وانسجة الخصي في ذكور الفئران. قسمت الفئران إلى ثلاثة مجاميع، احتوت كل مجموعة على تسعة فئران، أعطيت المجموعة الأولى (السيطرة) الماء المقطر عن طريق الفم، بينما أعطيت المجاميع الثانية والثالثة الدايمثويت بجرعات (0.1 و 0.2 مل دايمثويت/100 مل ماء مقطر) على التوالي ولمدة 20 يوم. عند نهاية فترة المعاملة، ستة فئران من كل مجموعة وزنت وقتلت، سجلت أوزان الخصي والبرابخ، وأنجزت الدراسات النسيجية على الخصي. أظهرت النتائج بأن الدايمثويت سبب انخفاض في وزن الجسم والبربخ، بالإضافة لانخفاض معنوي في أعداد الأجنة الميتة وأوزانها، كما أوضحت النتائج النسيجية بأن هذا المبيد سبب تغيرات ملحوظة في التركيب الدقيق لأنسجة الخصية تمثلت بوجود فجوات، عدم انتظام بالطلائية

ISSN (Print): 2413-5267
ISSN (Online): 2706-9966

التناسلية لبعض الأُنيبيبات المنوية وتغيرات الخلالية في البعض الآخر، بالإضافة لتأثيره على الأجنة. لذلك ينبغي بذل المزيد من الجهد لحماية صحتنا وبيئتنا من هذه المركبات الضارة والبحث عن طرق آمنة لمكافحة الآفات الحشرية.

الكلمات الدالة: الدايمثويت، الخصوبة، ذكور الفئران، الخصي.

1. Introduction

Pesticides are toxic chemicals used to control agricultural pests and insects causing health hazards to humans (Heikal *et al*., 2014). They are classified according to their toxicity, as extremely dangerous, highly dangerous, moderately dangerous, and slightly dangerous, and according to their chemical structure, they are classified into several groups, the most utilized of which are organochlorines, organophosphates, carbamates, and pyrethrins (Garcia *et al*., 2012). Organophosphate insecticides (OPI) constitute one of the most widely used classes of pesticides being employed for both agricultural and pest control (Selmi *et al*., 2014). They are also widely used in industry, medicine, and public health purposes (Yasin and Sharma, 2013). Many studies have shown that organophosphate caused changes in the central nervous system (CNS) characterized by excitation state of CNS and in some cases polyneuropathy, in addition to its effects on kidney, immune system and reproductive system (Yasin and Sharma, 2013), and some of the organophosphates are mutagenic and alter cell division (Gomes *et al*., 2008).

Dimethoate (DM) is one of the most used organophosphorus insecticides to control insects and house flies (Heikal *et al*., 2014), as it causes a health hazard to animals and humans because of its persistence in soil and crop (Sasi *et al.*, 2018b; and Ngoula *et al*., 2014). Several studies reported that dimethoate causes a decrease in sperm motility and an increase in the percent of abnormal sperm in the treated groups compared with control (Sasi *et al*., 2018 a & b; and Abdallah *et al*., 2010). Furthermore, dimethoate accelerated bone resorption activity in young rats during the suckling period after mother exposure to 40 mg/kg/day from days 0-10 after delivery (Amira *et al*., 2005). Reproductive toxicity of this pesticide on adult rodents of both sexes have been demonstrated altered level of serum gonadotropins and irregularities of the estrous cycle in females (Ngoula *et al*., 2014; and Kaur and Dhanju, 2005) and impairment of fertility, semen quality deterioration, altered testosterone levels and testicular degeneration in males (Farag *et al*., 2007). Exposure to dimethoate was also shown to have a direct effect on the ovary performance of adult female mice and accelerate bone resorptions activity in young rats during the suckling period following mothers' exposure. It also caused adverse reproductive effects on mating success, survival and growth of pups in male, and female mice. However, no teratogenic effects were observed (Farag *et al*., 2007). The aim of the current study was to evaluate the effect of direct exposure of dimethoate on the embryos and testicular architecture in male mice.

2. Materials and Methods

This experimental research on animals was performed with the ethical rules recognized by the Libyan National Committee for Biosafety and Bioethics.

ISSN (Print): 2413-5267
ISSN (Online): 2706-9966

2.1. Chemicals

DM was purchased from Soliman Khater market and used for experiments. The solutions were prepared weekly and maintained in dark glass bottles at room temperature (25°C).

2.2. Animals

27 Swiss albino mice, their ages ranged between 8-12 weeks and weighing between 22 and 27 grams were used in this study. The animals were bred and housed in the animal house at Zoology Department, Faculty of Science, University of Tripoli, they were placed in cages and were given water and food. They were kept under controlled temperature conditions (22±3°C) and a normal photoperiod of 12 hrs. dark/light.

2.3. Experimental Design

Animals were divided into three groups (nine mice per group): the second and third groups received daily gavage doses of (0.1 and 0.2 mL DM/100 mL distilled water) respectively for 20 days while the first group (control) was given distilled water orally.

2.4. Sample Collection

After the end of the treatment, six mice from each group were weighed and sacrificed by cervical dislocation. The testes and epididymis were dissected and weighed, while the rest of the mice from each group were used for a fertility test.

2.5. Histological Analysis

The testes of each animal were removed and placed in 10% formalin, dehydrated in a series of graded ethanol, cleared in xylene, and embedded in paraffin. Sections were cut at (5 µm), deparaffinized, hydrated and stained with hematoxylin and eosin. Slides were examined under a light microscope (Leica, Germany).

2.6. Fertility Test

Each male treated with dimethoate was placed with two untreated females. Once the vaginal plug was observed each female was caged separately. On the 18^{th} day of gestation, pregnant mice were killed by cervical dislocation and the embryos was removed from the uterus, the number of dead embryos was determined, and their body weight were recorded.

2.7. Statistical Analysis

Results were analyzed using SPSS (version 20), statistical significance were determined by one-way analysis of variance, followed by a Post hoc test for multiple comparisons. Data was expressed as; mean ± standard deviation (mean±SD). $P<0.05$ was considered statistically significant.

3. Result

3.1. Impact of Dimethoate on Body and Sex Organs Weights in Male Mice

The body and sex organs weights in the different groups have been monitored, so that investigate the impact of dimethoate on such parameters. The results in Figure (1) showed a decline in the body weight in mice treated with dimethoate compared to the control group,

ISSN (Print): 2413-5267
ISSN (Online): 2706-9966

this decreasing in body weight was more pronounced in animals that received the highest dose of dimethoate. A significant decrease (P<0.05) in the weight of epididymis was recorded in the mice which received the lowest dose of dimethoate, while No significant changes (P>0.05) were found in testes weights of treated mice when compared to the control group as shown in Figure (2).

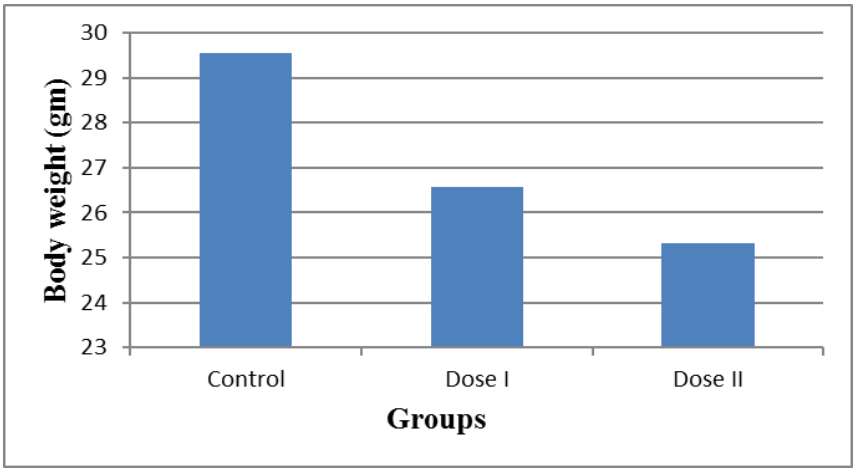

Figure 1. Effect of dimethoate on body weight in adult male mice.

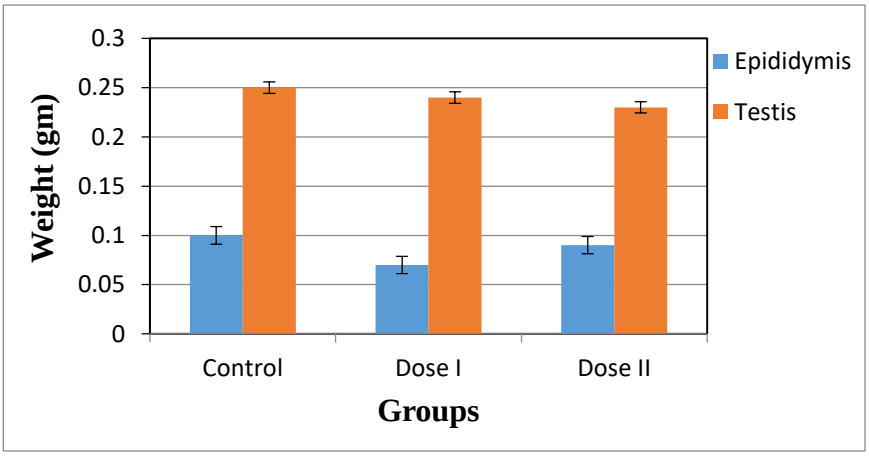

Figure 2. Effect of dimethoate on sex organs weight in adult male mice.

3.2. Effect of Dimethoate on Embryos of Untreated Females Impregnated by Treated Male Mice

The results in (Table 1) showed that dimethoate caused a significant increase (P<0.05) in the number of dead embryos and a significant decrease (P<0.05) in the body weight of embryos of untreated females impregnated by treated males. No significant difference (P>0.05) in body length of embryos of untreated females impregnated by treated males was recorded.

ISSN (Print): 2413-5267
ISSN (Online): 2706-9966

Table 1. Effect of dimethoate on embryos of untreated females impregnated by treated male mice.

Parameters / Groups	Body weight (g)	No. of dead embryos	Body length (cm)
Control	1.20 ± 0.07	0.1 ± 0.10	1.8 ± 0.16
Dose I, treated group	0.86 ± 0.06*	3.1 ± 0.29*	1.7 ± 0.19
Dose II, treated group	0.58 ± 0.05*	5.2 ± 0.37*	1.6 ± 0.10

*(P<0.05) significantly different from control group.

3.3. Histopathological Results

Histological analysis demonstrated a normal architecture of the seminiferous tubules with a normal pattern of spermatogenesis in the testes of the control (Figure 3a). in contrast, histopathological changes were detected in the testes of mice treated with dimethoate (Figures 3b-d). Testicular tissues of treated mice showed disorganization of germ epithelium, vacuoles between spermatogenic cells and inside them as well as degenerative changes in some seminiferous tubules, these effects were more pronounced in mice treated with the highest dose.

4. Discussion

Dimethoate was chosen in this study because it is widely used in our environment against many insect pests which infect the agricultural crops and is considered as one of the main causes of environmental pollution, so the purpose of this study was to assess the effect of dimethoate on testicular tissues and the embryos.

The study results indicated that administration of the recommended dose of dimethoate and its double led to a decrease in the bodyweight of treated mice, this result agreed with the study was conducted by Ngoula *et al*. (2014) who found a significant decrease in bodyweight of rats gavaged with dimethoate and also with the study of Verma and Mohanty (2009) who observed a decrease in the body weight of female mice exposed to dimethoate, this effect on the body weight may be attributed to anorexia or lack of appetite.

The present study also revealed that dimethoate caused harmful effects on testicular tissues, this was in agreement with previous studies were done by Sasi *et al.* (2018a) and Verma and Mohanty (2009), also with the study of Hess and Nakai (2000) who reported that OPIs cause tubular atrophy of testes. This toxic impact of dimethoate on testes is either by excessive production of free radicals which can impair cellular structure and function (Heikal *et al*., 2014), or by inhibition of gonadotropins secretion (Colborn, 2006).

ISSN (Print): 2413-5267
ISSN (Online): 2706-9966

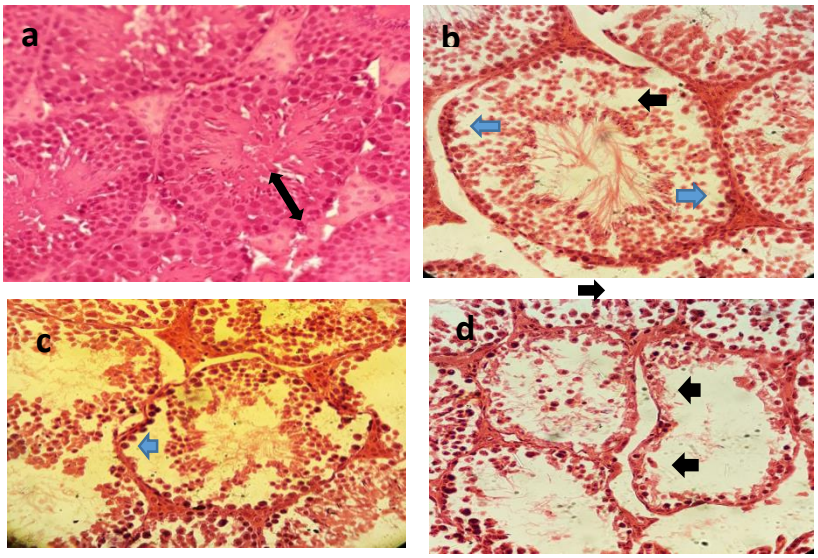

Figure 3. photomicrograph of a testicular tissue of the control group; **a)** showing normal seminiferous tubules and spermatogenesis (⬌). **b)** Mice treated with dimethoate at dose (0.1 mL DM/100 mL distilled water) showing vacuoles (⇨), loss of some spermatogenic cells (➡). **c)** Mice treated with dimethoate at dose (0.2 mL DM/100 mL distilled water) showing spermatogenesis arrest (⇦). **d)** Mice treated with (0.2 mL DM/100 mL distilled water) showing germ cell degeneration (➡) [H & E 40×].

The results of the current study demonstrated a significant increase in the number of dead embryos and a significant decrease in the mean bodyweight of the embryos, these results were in concord with previous studies (Sasi *et al*., 2018 a & b; and Farag *et al*., 2006). this detrimental effect of dimethoate on the embryos may be due to alteration in sperm DNA and chromatin (Salazar-Arredondo *et al*., 2008) because of oxidative damage induced by OPIs which occurs through the production of reactive oxygen species (ROS) (Mansour and Mossa, 2010).

5. Conclusion

The present results indicated that administration of dimethoate to male mice for 20 days has effects on testicular tissues and the embryos, so it is best to avoid excessive use of pesticides and search for new safe methods for insects' control.

References

Abdallah F.B., Slima A.B., Dammak I., Keskes-Ammar L., and Mallek Z. (2010). Comparative effects of dimethoate and deltamethrin on reproductive system in male mice. *Andrologia*, 42(3): 182-186.

Amira M.S., Hamadi F., Ghazi B., Kamel J., Emna A., Feriel E., Fadhel G., and Najiba Z. (2005). Effect of dimethoate on bone maturation og young rats during suckling period. *Pestic Biochem Physiol.*, 83(2-3): 132-139.

Colborn T. (2006). A case for revisiting the safety of pesticides: a closer look at neuro-development. *Environ. Health Perspect.*, 114(1): 10-17.

ISSN (Print): 2413-5267
ISSN (Online): 2706-9966

Farag A.T., Karkour T.A.Z., and El Okazy A. (2006). Developmental toxicity of orally administered technical dimethoate in rats. *Birth Defects Research*, 77(1): 40-46.

Farag A.T., El-Aswad A.F., and Shaaban N.A. (2007). Assessment of reproductive toxicity of orally administered technical dimethoate in male mice. *Reprod. Toxicol.*, 23(2): 232-238.

Garcia F., Ascencio S., Oyarzun J., Hernandez A., and Alavarado P. (2012). Pesticides: classification, uses and toxicity. Measures of exposure and genotoxic risks. *Journal of Research in Environ. Sci. Toxicol.*, 1(11): 279-293.

Gomes J., Lioyd O.L., and Hong Z. (2008). Oral exposure of male and female mice to formulations of organophosphorous pesticides: congenital malformations. *Hum. Exp. Toxicol.*, 27(3): 231-240.

Heikal T.M., Mossa A.T.H., Ibrahim A.W., and Abdel-Hamid H.F. (2014). Oxidative reproductive toxicity associated with cyromazine and chlorpyrifos in male rats: the protective effects of green tea extract. *Res. J. Environ. Toxicol.*, 8(2): 53-67.

Hess R.A. and Nakai M. (2000). Histopathology of the male reproductive system induced by the fungicide benomyl. *Histopath.*, 15(1): 207-224.

Kaur S. and Dhanju C.K. (2005). Biochemical effects of some organophosphorus pesticides on the ovaries of albino rats. *Indian J. Physiol. Pharmacol.*, 49(2): 148-152.

Mansour S.A. and Mossa A.H. (2010). Oxidative damage, biochemical histopathological alterations in rats exposed to chlorpyrifos and the antioxidant role of zinc. *Pest. Biochem. Physiol.*, 96(1): 14-23.

Ngoula F., Watcho P., Kenfack A., N'zouk Manga J., Fualefac Defang H., Pierre K., and Joseph T. (2014). Effect of dimethoate (an organophosphate insecticide) on the reproductive system and fertility of adult male rat. *American J. Pharmacol. Toxicol.*, 9(1): 75-83.

Sasi S.M., Al-Ghoul N.M., and Al-Shakshouki F.M. (2018a). Effect of Early Exposure of dimethoate on Reproductive Potential in Swiss Male Mice. *Syrian J. Agri. Res.*, 5(2): 212-221.

Sasi S.M., Sasi N.M., Abdulaziz A., Esa M., and Almsri M. (2018b). Effect of dimethoate (Insecticide) on reproductive function in adult male mice. *J. Libyan Studies*, 2(15): 1-12.

Salazar-Arredondo E., De Jesus Solis-Heredia M., Rojas-Garcia E., Hernandez-Ochoa I., and Quintanilla-Vega B. (2008). Sperm chromatin alteration and DNA damage by methyl-parathion, chlorpyrifos and diazinon and their oxon metabolites in human spermatozoa. *Reprod. Toxicol.*, 25(4): 455-460.

Selmi S., El Fazaa S., and Gharbi N. (2014). Oxidative stress and cytotoxic potential of anticholinesterase insecticide, malathion in reproductive toxicology of male adolescent mice after acute exposure. *Iran J. Basic Med. Sci.*, 17(7): 522-530.

Verma R. and Mohanty B. (2009). Early-Life Exposure to Dimethoate-Induced Reproductive Toxicity: Evaluation of Effects on Pituitary-Testicular Axis of Mice. *Toxicol. Sci.*, 112(2): 450-458.

Yasin M. and Sharma P. (2013). Effect of dimethoate 30EC on some hematological parameters of albino mice following an oral exposure. *Int. J. Recent Scientific Res.*, 4(9): 1323-1326.