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Original article

Effect of Dimethoate Insecticide on Female Mice and Their Fetuses

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ABSTRACT

Background and objectives. *Organophosphate insecticides (OPIs) including dimethoate are used in agriculture to control the insect pests, increasing the productivity, their frequent use has resulted in detrimental effects on biological systems and public health. This study aimed to evaluate the effect of dimethoate (DM) on fetuses, DNA concentration in ovarian tissues and levels of estradiol and progesterone in female mice.* ***Methods***. *Female mice were divided into three groups: Group I, serve as control and was given distilled water intraperitoneally, while Group II and III were given (0.1- & 0.2-ml DM/100 ml distilled water) respectively for 7 days. Two treated females were housed with one untreated male for mating. After 7 days of the last dose, 6 female mice of each group were weighed and killed. The ovaries, uteri and liver were extracted and weighed, and ovarian hormones were measured. Also, DNA concentration in each ovary was determined.* ***Results***. *The results showed a significant decrease in the weights of the liver and ovaries, the level of estradiol hormone, and DNA concentration in the ovary of treated mice as compared to those from the control group. Also, significant changes were observed in the weights of fetuses as well as the number of live fetuses.* ***Conclusion***. *We found that administration of DM intraperitoneally to female mice for 7 days has influences on ovary weight, estradiol levels, and DNA concentration as well as its impact on the fetuses, therefore, it is better to use safer methods for control the pests and avoid increased use of pesticides.*

Keywords: Dimethoate, Female Mice, Ovary Hormones, DNA Concentration, Fetuses.

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الخلفية والأهداف. تُستخدم المبيدات الحشرية الفوسفورية العضوية (OPIs) بما في ذلك الديمثويت في الزراعة للسيطرة على الآفات الحشرية، وزيادة الإنتاجية، وقد أدى استخدامها المتكرر إلى آثار ضارة على النظم البيولوجية والصحة العامة. هدفت هذه الدراسة إلى تقييم تأثير الدايمثويت (DM) على الأجنة وتركيز الحمض النووي في أنسجة المبايض ومستويات الاستراديول والبروجستيرون في إناث الفئران. **طُرق الدراسة.** تم تقسيم إناث الفئران إلى ثلاث مجموعات: المجموعة الأولى، كانت بمثابة مجموعة السيطرة وتم إعطاؤها ماء مقطر داخل التجويف الصفاقي، في حين أعطيت المجموعة الثانية والثالثة (0.1 و 0.2 مل DM / 100 مل ماء مقطر) على التوالي لمدة 7 أيام. تم وضع الإناث المعاملة بالدايمثويت مع الذكور غير المعاملة بنسبة (2 :1) للتزاوج. بعد 7 أيام من إعطاء الجرعة الأخيرة، تم وزن وقتل 6 إناث من كل مجموعة، تم استخراج المبايض، الرحم والكبد ووزنها وقياس هرمونات المبيض، كما تم تحديد تركيز الحمض النووي (DNA) في كل مبيض. **النتائج.** أظهرت النتائج انخفاضاً معنوياً في أوزان الكبد، المبايض، مستوى هرمون الاستراديول وتركيز الحمض النووي في مبايض الفئران المعاملة مقارنة بالمجموعة الضابطة، كما لوحظت تغيرات كبيرة في أوزان الأجنة وكذلك عدد الأجنة الحية. **الخاتمة.** لقد وجدنا أن حقن DM داخل التجويف الصفاقي في إناث الفئران لمدة 7 أيام له تأثير على وزن المبيض، مستويات هرمون الاستراديول، تركيز الحمض النووي بالإضافة لتأثيراته على الأجنة، لذلك، من الأفضل استخدام طرق أكثر أمانا لمكافحة الآفات ومحاولة التقليل من الاستخدام المتزايد للمبيدات الحشرية.

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INTRODUCTION

Pesticides are one of the most detrimental chemicals liberated in the environment in an unplanned manner [1]. They have negative effects on animals and humans [2]. These effects may occur directly through bioaccumulation or indirectly via the food chain. Pesticides also have the ability to inhibit enzyme activity causing oxidative stress [3]. Organophosphate insecticides (OPIs) are used in agriculture to control the insect pests, increasing the productivity and quality of agricultural products. However, their frequent use has resulted in detrimental effects on biological systems and public health [4].

OPIs have been found to induce histopathological and biochemical changes in different tissues like liver [5], kidney [6], pancreas [7] and testes in mice [8]. Some studies reported that OPIs are potent inhibitors of acetyl cholinesterase [9] and may cause oxidative stress leading to the production of large amounts of free radicals [10, 11]. These free radicals are toxic and intensively combined with macromolecules causing damage to various organs [12]. Dimethoate (DM) is an organophosphorus pesticide, that is commonly used to control pests that affect fruit, vegetables and agricultural crops [13, 14]. However, DM products can be toxic to higher vertebrates through inhalation, ingestion and dermal absorption [15], and can cause blockage of neuromuscular transmission [16].

Several studies indicated that exposure to DM can led to defects in fetuses of female mice [16], as well as infiltration in the liver of chick embryos [17]. It can also have an effect on many organs such as the brain [18], and the ovary [19]. In addition, DM has been found to induce DNA damage in mice bone marrow [20]. Histopathological lesions of DM were observed in seminiferous tubules of rats and mice [21, 22], as well as in the liver and kidney [23].

There are few studies on the effect of DM on female mice; Therefore, the aim of this work was to investigate the impacts of DM which is extensively used in many agricultural areas in Libya on ovary hormones, fetuses and DNA concentration in ovarian tissues

METHODS

Chemicals

DM (40% EC, good quality, Germany) was purchased from Soliman Khater market (Tripoli/Libya) in a plastic bottle (250 ml) and it was used for preparing the required doses. The working solution was prepared weekly and maintained in dark bottles at room temperature (25 c°).

Experimental animals and treatments

Thirty-six female mice weighing (17-20 g) were used in this study, all animals were housed in standard plastic cages at the animal house, Faculty of Science, University of Tripoli. Mice were divided into three groups (n=12 per group) and treated as follows: Group I: served as control and received distilled water intraperitoneally for 7 days. Group II and III: given intraperitoneally (0.1- & 0.2-ml DM/100 ml distilled water) respectively, for 7 days.

Sampling

After 7 days of the last dose, 6 mice from each group were weighed and killed, ovaries, uteri and liver were extracted and weighed, then kept in 10% formalin.

Measurement of Progesterone and Estradiol

Blood samples were collected from the facial veins of all mice, after that, the hormones were measured by ELISA technique.

Measurement of DNA concentration in ovarian tissues

Ovaries from the control and treated groups were kept at (- 80 C°), then, DNA was extracted using QI Aamp DNA mini kit (Qiagen), after that, DNA concentration was measured by Nano drop device.

Fertility test

For mating, treated females were put with untreated males (2:1) in a cage overnight. Then, mating was confirmed by vaginal plug and they were marked as gestation day zero. On 18 day of gestation, pregnant mice were killed and the fetuses were removed from the uterus. The number of live fetuses was determined and their weight and length were recorded.

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Statistical analysis

The obtained data were analyzed using SPSS (version 20). One-way analysis of variance (ANOVA) and post hoc Duncan's test were used for comparisons between treated groups and the control. The results were expressed as mean ± standard deviation (mean ± SD). $P < 0.05$ was considered statistically significant.

Ethical approval

Ethical approval of dealing with mice in this study was carried out according to the guidelines of the Ethics Committee of University of Tripoli (Ref No; SREC 16-2022).

RESULTS

No mortality was observed among the animals during the experimental.

Effect of DM on body and some organs weight.

As can be seen from Table 1, there is no significant difference (P>0.05) in the mean body and uterus weight in treated mice as compared to the control. A significant decrease (P<0.05) in ovary and liver weights was observed in the treated groups when compared with the control group

Table 1. Body and some organs weight of female mice exposed to DM.

Groups	Body weight	Uterus	Ovary	liver
G I	16.6 ± 6.15	0.02 ± 0.01	0.09 ± 0.07	0.78 ± 0.39
GII	13.02±4 .03	0.03 ± 0.02	0.04± 0.03*	0.65± 0.24*
G III	10.02± 2.61	0.02 ± 0.01	0.02± 0.02*	0.32± 0.16*

**(p<0.05) significantly different from control, values are expressed as (mean ± SD).*

Effect of DM on progesterone and estradiol in female mice.

The results of this study revealed a significant decrease (P<0.05) in estradiol levels in the treated groups compared with the control, and non-significant alterations were found in progesterone levels between the treated groups and control Table 2.

Table 2. Progesterone and Estradiol levels in female mice exposed to DM.

Groups	Progesterone(ng/ml)	Estradiol (pg/ml)
G I (control)	1.04 ± 1.00	45.67 ± 24.12
GII	0.67 ± 0.96	30.32 ± 25.89*
GIII	1.01 ± 1.32	23.92 ± 3.83*

**(p<0.05) significantly different from control, values are expressed as (mean ± SD).*

Effect of DM on DNA concentration extracted from ovaries.

The findings of the current research showed a statistical difference ($P < 0.05$) in DNA concentration between the treated groups and the control group Table 3.

Table 3. DNA concentration extracted from the ovary in female mice exposed to DM.

Concentration	*GI*	*GII*	*GIII*
DNA (ng/ul)	215.83± 210.12	105.24± 9.90*	98.84 ± 80.46*

**(p<0.05) significantly different from control, values are expressed as (mean ± SD).*

Effect of DM on the fetuses

The obtained results showed a significant difference (P<0.05) in the average body weight of embryos and the average number of live fetuses in treated groups as compared with the control. No significant changes in the body length of fetuses were observed Table 4.

Table 4. Effect of DM on the fetuses.

Groups	Body weight (g)	No of live fetuses	Body length (cm)
G I	1.23 ± 0.17	9.66 ± 0.07	1.95 ± 0.16
GII	1.15 ± 0.21*	6.10 ± 0.53*	1.80 ± 0.14
G III	1.07 ± 0.21*	5.80 ± 0.50*	1.64 ± 0.13

**(P<0.05) significantly different from control, values are expressed as (mean ± SD).*

DISCUSSION

This study was carried out to assess effects of DM on ovary hormones, fetuses and DNA concentration in ovarian tissues.

The results demonstrated that DM had no effect body and uterus weights in treated females compared to the control females. This finding disagreed with previous studies which reported that DM caused a significant decline in the body weight of treated rats [24, 25] and mice [26, 27]. Additionally, a previous study found that treated

rats with DM caused a marked decline in the weight of the uterus [14]. This difference between the studies may be attributed to the treatment period, gender of the animals or the route of administration. DM reduced ovary and liver weights in treated females when compared to females in the control group. Similar results were obtained by Heikal *et al* [27] who found that treated rats with DM for 28 consecutive days led to a decrease in liver weight. EL-Damaty *et al* [26] elucidated that treated rats with DM at a dose of (40 mg/kg daily) for 30 days created a decrease in liver weight. Abouamer *et al* [14] stated that Administering DM orally to pregnant mice in varying doses led to a decrease in ovary weight.

DM revealed a significant decrease in estradiol levels in the treated groups compared with the control, and non-significant alterations in progesterone levels between the treated groups and control. Previous research indicated that diazinon (an organophosphorus pesticide) resulted in a reduction in the level of progesterone and did not produce any effect on estradiol hormone [28].

Another study demonstrated that the administration of malathion to rats led to a significant decline in the level of progesterone [29]. ELham *et al* [12] found that female rats treated with malathion for 14 days led to a decrease in levels of estrogen hormone at the doses (10 & 20 mg/kg), while progesterone hormone showed a significant drop at all the used doses.

A study by Zhou et al [30] stated that cypermethrin administration orally to female mice for 3 months caused disrupted the balance of estradiol and progesterone hormones. This reduction in estradiol levels may be due to damage to the histological structure of the ovary [29].

The findings of the current research showed a statistical difference in DNA concentration between the treated groups and the control group. Similar results were done by Dutta and Maxwell [31] and Kaur & Kaur [32] who revealed that organophosphorus pesticides can affect DNA. Another study demonstrated that acute and chronic exposure of rats to chlorpyrifos, methyl parathion, and malathion caused a marked DNA damage in tissues of the liver, brain, kidney and spleen [33]. Wang *et al* [34] elucidated that diazinon exposure led to DNA damage in granulosa cells in the ovary. Alam *et al* [35] stated that fenitrothion (an organophosphorus pesticide) raised the level of oxidative DNA damage in the cerebrum and spleen of male rats. Nazam *et al* [36] found that DM caused DNA damage led to the apoptosis of leukocytes. OPIs can generate large amounts of free radicals in a short duration which in turn cause oxidative damage to DNA bases and telomeric DNA [33, 37].

The obtained results showed a significant difference in the average body weight of embryos and the average number of live fetuses in treated groups as compared with the control. No significant changes in the body length of fetuses were observed. These consequences were in agreement with the results of previous studies indicating that DM caused a reduction in the body weight of fetuses [14, 16, 21]. Other studies have showed that treatment of mice with DM led to a significant decline in the number of live fetuses [8, 38]. Sasi *et al* [39] illustrated that the administration of DM to pregnant mice during the organogenesis period did not produce any significant change in the mean body weight of fetuses. Ducolomb *et al* [40] indicated that diazinon and malathion have toxic effects on embryo development. This effect of DM may be due to its transferring via the placenta during gestation [15] and the production of free radicals which lead to embryos growth retardation and cell death [41].

CONCLUSION

According to the results of this study, we can say that administration of DM intraperitoneally to female mice for 7 days has influences on ovary weight, estradiol levels, and DNA concentration as well as its impact on the fetuses, therefore, it is better to use safer methods for control the pests and avoid increased use of pesticides.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare

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