



The Protective Role of Wheat Germ Oil against Adverse Effect of Deltamethrin on Reproductive Aspects of Male Albino Rats

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ABSTRACT

Deltamethrin (DLM) is a synthetic pyrethroid type-II known to cause many adverse effects in experimental animals such as endocrine disruption, reproductive toxicity, and negative impact on gametogenesis. The present study was conducted to evaluate the protective role of wheat germ oil against reproductive toxicity induced by Deltamethrin (DLM) in male albino rats through evaluation of semen picture, measuring the testosterone levels, DNA fragmentation percentage and pathologic changes of the testes. Thirty male rats were divided into three groups (10 each); the first group (C) was administered with normal saline, the second group was given (DLM) at a dose of (6 mg/kg. B.W. of rats) and the third group (DLM+WGO) was treated with wheat germ oil (1.5 ml/Kg. B.W. of rats), and after one h DLM (6 mg/kg. B.W. of rats) was administered. All treatments were orally given using stomach gavage for 30 days. The rats were left for 65 days of treatment to complete time of one spermatogenesis and maturation of sperms in epididymis then sacrificed and blood samples were collected, testes and epididymis were removed, weighted, and examined histopathologically. DLM administration significantly decreased the weight of testes and adversely affected the semen quality. Testosterone levels were significantly reduced. There is a significant elevation in the DNA laddering percentage in the DLM group compared to the C group. Histopathological examination of the testes revealed that DLM induced changes in the form of distorted seminiferous tubules with intraluminal sloughing degenerated spermatogenic cells. Also there is marked interstitial edema and congestion. The co-administration of DLM with wheat germ showed improvement in weight of testes, semen pictures, testosterone level and a significant reduction in the DNA laddering percentage compared to DLM group. It can be concluded that, the wheat germ oil can mitigate the adverse effects of DLM on male fertility, and leads to improvement of male reproductive performance.

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INTRODUCTION

Many insecticides are extensively used in agriculture for insect control. For example, pyrethroid insecticides have been increasing and replacing organophosphorus compounds hence, animals and human exposure to these insecticides were increased (Saber and Wael (2012).

DLM is a synthetic type II- pyrethroid insecticide. It is widely used by farmers as an ectoparasiticide in animals against (ticks, flies, fleas, and mites) and as an insecticide in crop production to protect crops, fruits, and, vegetables against pests such as ants, weevils, mites, and beetles (Tu *et al.*, 2007 and Mehlhorn *et al.*, 2011).

The uses of pesticides were known to impair the reproductive proficiency of males. Deltamethrin

exposure is known to cause many adverse effects in experimental animals such as endocrine disruption and reproductive toxicity. **Makarov et al., (2020)** found that, deltamethrin possess a negative impact on gametogenesis in mice.

Chronic exposure of deltamethrin to environmentally relevant levels can have detrimental effects on vital organs (**Anita, et al., 2018**) and causes varying degrees of toxicity (**Qirong et al., 2019**). Natural products are considered one of the common means of alternative and complementary medicines. Several extracts of these products exhibit protective effects. Wheat germ oil (WGO) is a special product characterized by its higher content of vitamin-E. Therefore, it is used in many applications like cosmetics, pharmaceuticals, and dietary supplements. In addition, WGO is rich in bioactive compounds, such as tocopherols, carotenoids, omega-3 fatty acids, and phytosterols (**Nurhan, 2009**).

Many studies reported the protective role of WGO as a rich source of vitamin E and powerful antioxidant against hepatotoxicity, nephrotoxicity, gastrotoxicity and reproductive impairment induced by several drugs. Therefore, this study was carried out to investigate the potential protective role of WGO against DLM induced reproductive toxicity in male albino rats.

MATERIAL AND METHODS

The study follows internationally and institutionally humane animal treatment guidelines and complies with relevant legislation in Egypt. It was performed in accordance with the Institutional Animal Care and Use Committee of Beni-Suef University ethical guidelines (BSUIACUC).

Chemicals

Deltamethrin (Butox[®] 5%) was purchased from Arab company for Chemical ind., Egypt. WGO was purchased from El-Captain Company, Egypt. The chemical composition of the WGO sample was analyzed using Gas Chromatography (GC)-Mass Spectrometer (Agilent Technologies 7890A GC system equipped with a 5975 Inert MS with triple Axis Detector, Germany), as illustrated in (Table 1).

Animals

Thirty male albino rats, weighing 200-250 g were housed in groups (3/cage), the dimensions of cages were (47×25×21) cm. Rats were accommodates for 2 week before the onset of the study in the Physiology department, Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt. They kept under controlled laboratory conditions of a normal light-dark cycle (12h), in well-ventilated room, 45±5% humidity, and 25±2°C temperature. The

rats were watered *ad libitum* and fed on a commercially prepared pellets throughout the study.

Table 1: The chemical compositions of wheat germ oil sample by using (GC)--mass spectrometry

No	Name of the compound	Retention time (RT)	Area %
1	Benzene, 1-methyl-3-(1-methylethyl)	6.593	0.08
2	Furan	10.701	0.41
3	Nonadienal2,4	11.027	0.70
4	di-tert-Butyl-m-cresol4,6	13.614	0.03
5	Glycerol tricaprylate	18.083	0.79
6	n-Hexadecanoic acid	18.558	10.69
7	Dodecanoic acid	18.752	0.18
8	Dodecane	19.170	0.09
9	Adipic acid, isoheptyl 3-oxobut-2-yl ester	19.353	0.02
10	Ascorbic acid	19.542	0.27
11	Heneicosane	19.788	0.30
12	Heptadecane, 2,6,10,15-tetramethyl	19.897	0.25
13	9,12- Octadecadienoic acid	20.543	56.6
14	cis-Vaccenic acid	20.732	7.53
15	3-Eicosene	21.155	0.93
16	Vitamin E	21.928	0.07
17	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl	24.857	1.30
18	beta.-Tocopherol	24.960	0.16
19	gamma.-Tocopherol	26.168	6.05
20	Squalene	29.338	0.10
21	dl.-alpha.-Tocopherol	30.717	3.22
22	Campesterol	33.040	0.40
23	gamma.-Sitosterol	33.114	0.48

Experimental design

After two-week acclimatization period, rats were divided randomly into three different groups; (10 each). The first group (C) was the control group and administered normal saline. The second group was given (DLM) at a dose of (6mg /kg.B.w.) according to **El Banna et al., (2016)**. The third group (DLM+WGO) was treated with WGO (1.5 ml/Kg.B.w.), **Karabacak et al., (2011)** and after one h DLM (6mg /kg. B.w.) was administered.

All treatments were orally given using intra-gastric gavage needle, once daily for 30 days. The rats were left for 65 days to complete the time of at least one spermatogenesis and sperms maturation.

Blood samples and Hormonal assay:

At 65 days of treatment, animals were weighed and anesthetized by diethyl ether for retro-orbital venous plexus blood collection using clean micro-capillary tubes. Blood samples were left at 37°C until clotting, centrifuged for 15 min at 3000 rpm to obtain serum then, frozen at -20°C for further processing. The serum testosterone level was estimated using Enzyme immunoassay kit for the

determination of testosterone quantitatively (Chemux Biocience, Inc, South San Francisco, CA94080, USA) (Chen *et al.*,1991).

Testes weights:

All rats were euthanized by dislocation of cervical vertebrae, and then the testes were dissected out and weighted. The relative weight of testes was calculated using the following equation (Hala *et al.*, 2012)

$(\text{Organ weight (g)} \times 100) \div \text{The corresponding total body weight.}$

Semen evaluation:

Semen samples were obtained by cauda-epididymis maceration, which diluted with Tris buffer solution, and evaluated microscopically within 2-4 min. for sperm motility (Narayana *et al.*, 2005). In addition, semen smears were made and stained with 1% eosin to measure sperm count by using a haemocytometer. Finally, semen film was prepared and stained by Eosin-Nigrosin stain, then examined under a light microscope to determine sperm abnormalities. The abnormalities of sperm were classified according to their origin into; primary abnormalities (double head, dwarf or giant head, double tail and/or coiled tail) and secondary abnormalities (wavy and/or bent tail, detached head) (Bearden and Fuqucy, 1980).

DNA Fragmentation:

The DNA laddering by Diphenylamine was assessed in testicular tissue according to the method described by Hassanen *et al.*, (2020). And its percentages were calculated as: "DNA laddering %= [OD₆₀₀ of the supernatant/ (OD₆₀₀ of the supernatant S+OD₆₀₀ of the pellet)] X 100"

Agarose gel electrophoresis of the fragmented DNA:

By using a DNeasy kit (Qiagen), the isolation of fragmented DNA from the supernatant portion was done. The fragmented DNA electrophoretic patterns were checked in 2% agarose gel that stained with ethidium bromide (Hassanen *et al.*, 2020).

Histopathological investigations:

Testes from all rat groups were histopathologically investigated according to the method of Banchroft and Gamble (2008). First, testes were fixed with neutral buffered formalin 10 %, washed in water, dehydrated in alcohol ascending grades, then in xylene. Then, the samples of testis were embedded in paraffin to prepare paraffin sections (5 µm) and stained with hematoxylin and eosin.

Statistical analysis:

Data were compared with one-way (ANOVA) analysis of variance using SPSS version 20 software. Tukey's test *Post hoc* analysis was carried out. *p* < 0.05 were considered significant.

RESULTS

Sperm picture:

Regarding sperm picture, data in Table (2) showed that administration of DLM in male rats for 30 days, significantly decreased sperm concentration, individual motility % as well as, it caused an elevation in the total abnormalities % compared to the control group. On the other side, the administration of WGO combined with deltamethrin successfully removed the toxic effect induced by deltametherin. The DLM group showed a significant decreased in the relative weight of testis compared to that of the control group, . In contrast, WGO group exhibited a considerable increase compared to the DLM group, Table (3).

Serum testosterone levels

Concerning to the effect of DLM administration on serum testosterone level, it was shown that there was a significant decrease in its level compared with control. However, treatment with WGO caused a numeral but not significant increase in testosterone level compared with DLM group Table (3).

The microscopic examination of testis sections in the control group appeared normal (Fig.2.a), while testis of the DLM group revealed a thick tunica albuginea , distorted seminiferous tubules, intraluminal sloughing and degenerated spermatogenic cells (Fig.2.b). Moreover, there is marked interstitial edema and congestion (Fig.2.c). However, treatment with WGO resulted in the preservation of the germinal epithelium, In addition, there were some disrupted seminiferous tubules detected with few degenerated cells and minimal interstitial edema (Fig.2.d).

DNA fragmentation percentage

DNA laddering percentage significantly elevated in the DLM group compared to that of the control group. The co-administration with WGO showed a significant reduction in DNA laddering percentage compared to that of the DLM group. (Table, 4) and (Fig.1).

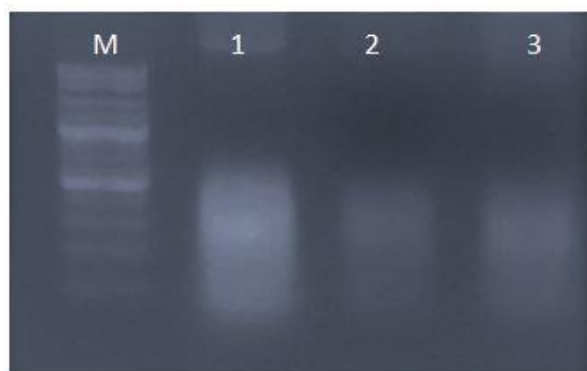


Fig. 1. Fragmented DNA on 2% agarose gel electrophoretic mobility. Lane M: 100 bp DNA ladder; lane 1: C group; lane 2: DLM group; Lane 3: DLM + WGO group.

Table 2: Sperm picture of control and treated rats

Treatment	Sperm conc. × 10 ⁶ /ml	Individual motility %	Abnormalities %
Control group	2.8 ± 0.27 ^a	80.0 ± 2.90 ^a	32.67 ± 2.8 ^a
Deltamethrin group	1.3 ± 0.26 ^b	38.33 ± 4.40 ^b	54.0 ± 3.6 ^b
WGO+ Deltamethrin group	2.43 ± 0.20 ^a	66.67 ± 7.27 ^a	39.67 ± 5.82 ^a

Results are demonstrated as (mean ± SE), values with different superscript letters within the same column; were significantly different ($p \leq 0.05$).

Table 3: Relative testis weight (g) and serum testosterone level (ng/ml) in controls and treated rats

Treatment	Relative weight of testis (g)	Testosterone (ng/ml)
Control group	0.83 ± 0.027 ^b	4.75 ± 0.26 ^a
Deltamethrin group	0.61 ± 0.051 ^a	1.96 ± 0.06 ^b
WGO+ Deltamethrin group	0.81 ± 0.32 ^b	3.88 ± 1.03 ^{ab}

Results are demonstrated as (mean ± SE), values with different superscript letters within the same column; were significantly different ($p \leq 0.05$).

Table 4: DNA fragmentation percentages:

	Control group	Deltamethrin group	WGO+ Deltamethrin group
DNA fragmentation %	22.59 ± 0.55	65.9 ± 0.43 ^{**}	41.6 ± 0.43 [*]

Results are demonstrated as (mean ± SE)

* Superscript means significant differences versus control at $P \leq 0.05$.

** Superscript means significant differences versus control at $p \leq 0.01$.

DISCUSSION

In this work, DLM exposure at (6 mg / kg b.w.) for 30 days decreased the concentration and motility of sperm; therefore, there was an increase in the total abnormalities %. These results were similar to those found by (El Aziz et al., (1994); they recorded that DLM administration to rats at (1, 2 mg/kg b.w.) for 65 sequential days could decrease sperm parameters. Also (Al-Sarar et al., (2014) who reported that synthetic pyrethroids on the whole

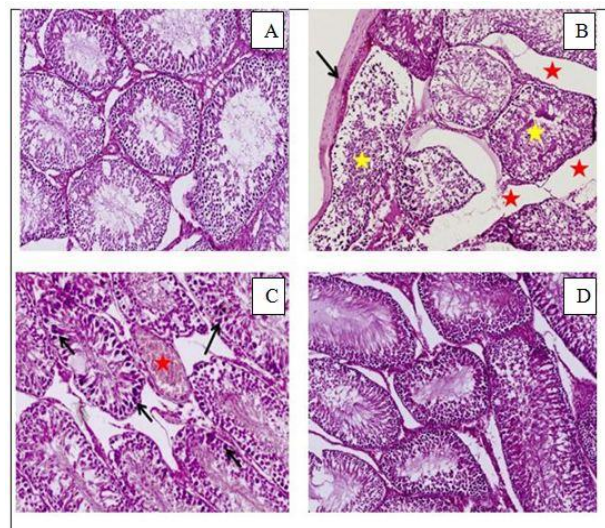


Fig. 2: **A:** Light microscopy of the control group testicular section clarified that testicular parenchyma is consisted seminiferous tubules that are closely packed, with organized spermatogenic cells & regular lumen. The minimal Interstitial space was noticed. (H&E stain ×100). **B:** Light microscopy of the testicular section from the DLM group revealed thick tunica albuginea (black arrow) with distorted seminiferous tubules showed interrupted spermatogenesis with intraluminal sloughing degenerated spermatogenic cells (yellow stars) , marked widening Interstitial space (edema)& decrease number of Leydig cells (red stars) .(H&E stain ×100). **C:** Light microscopy of testicular section from DLM group showed disrupted seminiferous tubules showed marked decreased spermatogenic cells with prominent sertoli cells with pyramidal nuclei (black arrows). Noting marked Interstitial edema& congestion (red star) .(H&E stain ×200). **D.** seminiferous tubules in WGO plus DLM showing preservation of the germinal epithelium with regular empty lumen, some disrupted seminiferous tubules were detected with few degenerated cells. The minimal Interstitial edema was also noticed (H&E stain ×100).

impairs sperm characteristics with sperm count reduction which may be due to an adverse effect of DLM on spermatogenesis. DLM at (5 mg/kg body weight/day) was caused alteration in total sperm density, motility, and morphology in spermatozoa of mice (Ben Slima et al., 2017) and caused decreases in sperm count, sperm motility, reproductive organs weight and level of testosterone (Poonam et al., 2018).

DLM showed a significant decrease in the relative weight of testis compared to the control group. In addition, the histopathological studied indicated that the DLM group revealed disrupted seminiferous tubules and, showed a marked decrease number of spermatogenic cells with marked Interstitial edema and congestion.

These finding were similar to the result reported by **Saber and Wael (2012)**. They found that deltamethrin caused a decrease in testes and body weights in treating animals with significant decreases in sperm motility and sperm concentrations. Also, DLM induced spermatogenic cells degeneration, blood vessels congestion, and Leydige cells destruction. Furthermore, it significantly reduced the seminiferous tubules diameters and their germinal epithelium heights. **Ben Slima et al., (2017)** revealed that DLM made severe seminiferous tubules alterations, germ cells sloughing, germ cell cytoplasm vacuolization, and the spermatogenic cells disruption when compared to the control group. These results was supported by **Hozyena et al., (2020)** when they reported that the toxic effects induced by DLM were confirmed by alterations in histology, represented by a significant reduction in spermatogenesis and the diameter of the seminiferous tubules.

DLM treated group was shown a significant decrease in serum testosterone level when compared with the control group. Our results were in line with those been reported by **Ben Slima et al., (2017)** who showed that oral administration of DLM caused a decrease in plasma testosterone levels and resulted in reproductive system toxicity in male mice. The current results were similar to **Hoda et al., (2018)**; they showed that testosterone level, motility, and spermatozoa count were significantly decreased, and increased abnormal spermatozoa in the DLM group when compared to the control group. In addition, DLM caused reduced spermatogenesis progenitor cells, degenerative effect on testicular tissue, and reduced the seminiferous tubules epithelial height and diameter. Also, the results obtained agreed with that obtained by **Hozyena, et al., (2020)**, as DLM caused a significant reduction in body weight and sperm count, motility, sperm abnormalities (%), viability percent and levels of serum testosterone.

The current results showed that rival administration of DLM with WGO to male rats caused an improvement of semen quality and quantity. In addition, significant increases in the testes relative weight, testosterone serum levels of and improve the cellular damage in the testes as a result to exposure to DLM. These improvement effects of WGO may be due to its high content of vitamin E, α -tocopherol and other antioxidants as proven by GC-MS analysis.

Similar to **El Banna et al., (2016)**, these findings reported that vitamin E practice a protective effect against toxicity of testis induced by DLM in male rats. Also, **Jedlinska et al., (2006)** concluded that vitamin E could protect DNA of sperm from oxidative stress and enhance male fertility and spermatogenesis due to its potent antioxidant activity. Furthermore, **Kumar et al., (2019)** revealed that α -tocopherol shows immune-protective effects in splenic and thymic apoptosis induced by DLM through inhibiting the oxidative stress and caspase dependent apoptogenic pathways. Next, **Dina et al., (2020)** reported that WGO showed a protective effect on body weight changes and Pregabalin-induced reproductive toxicity by affecting caspase3 and leptin expression.

This study provides testimony that DNA laddering is increased due to the DLM exposure, suggesting the serious effects of these materials on the testes (**Abdel Aziz et al., 2018**). Moreover, wheat germ oil can improve the damage of testicles caused by heavy metal and heat (**Khalaf et al., 2017**). Thus, Wheat germ oil maintains normal testicular functions and provides protective effects against testicular injury.

CONCLUSION

Deltamethrin negatively affects the reproductive function. While, WGO has a protective effect against DLM reproductive toxicity as it caused improvement of semen quality and quantity, increases the serum levels of testosterone, and improves the cellular damage in the testes caused by deltamethrin. WGO can be used to overcome the adverse effects of DLM.

Statement of conflict of interests

All authors declare there is no conflict of interest.

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