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Research Article

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Reproductive Toxicity of Deltamethrin in Male Rats and the Protective Role of Vitamin E

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Abstract This study was performed to assess the effect of deltamethrin (DLM) insecticide on male fertility in rats and to examined the possible protective role of vitamin E. Twenty five sexually mature male rats were *randomized* into 5 groups; one group was kept as a negative control. In the remaining 4 groups, DLM was orally administered at doses *6 and 3 mg* kg^{-1} *(corresponding to 1/10 and 1/20 of* LD_{50}*) b.wt* alone and / or in combination with vitamin E, for 65 days. Blood samples were withdrawn for determination of testosterone, FSH and LH in the serum. Semen analysis, Sex organs weight, antioxidant (superoxide dismutase, SOD; Glutathion peroxidase and Catalase, CAT) enzyme activities in testicular tissue and histopathological changes in testes were evaluated. The results showed that concurrent administration of vitamin E with DLM improved the relative weight of testes and increased sperm cell concentration and percentage of sperm motility and viability. There were also significant increases in serum testosterone, FSH and LH levels and antioxidant enzymes activity in testes, associated with amelioration *of testicular degenerative changes.*In conclusion, vitamin E exerts a protective effect against testicular toxicity induced by DLM in male rats.

Keywords Deltamethrin, Vitamin E, Male fertility, Sperms, Testosterone

Introduction

Infertility is one of the major health problems and about 30 % of this problem is due to male factors [1]. Several factors can interfere with the process of spermatogenesis and reduce sperm quantity and quality and so lower the male fertility. Many diseases and conditions such as coronary heart diseases, diabetes mellitus, chronic liver diseases, chronic smoking, alcohol intake, prolonged exposure insecticide contaminants, air pollutants and intake some drugs have deleterious effects on the process of spermatogenesis [2-5]. Moreover, more than 90% of male infertility cases are due to low sperm counts, poor sperm quality and both. The remaining cases of male infertility can be caused by a number of factors including anatomical problems, genetic defects [6] and hormonal imbalance [7]. Because oxidative stress is evoked by many chemicals including some pesticides, the antioxidants can reduce the deleterious effects of insecticides and reduce the damaging effect of oxidative stress on testes [8-9].

Vitamin E (α- tocopherol) regulates oxidation processes in the body as it is a powerful antioxidant. Previous studies showed that intake of vitamin E could normalize the damaging effect of oxidative stress induced by oxygen free radicals [8, 10]. Previous studies have reported that intake of vitamins E and C can protect sperm DNA from oxidative stress in rat testes [11] and antagonize testicular toxicity caused by a pyrethroid insecticide (Lambda - cyhalothrin) as reported by [12].

Pyrethroids are pesticides with high selectivity for insects. Experimental evidence suggests the pyrethroid molecule may bind to sex hormone binding globulin (SHBG) *in vitro*. Chronic exposure to pyrethroid may result in disturbances in hormonal effects relating to androgen action. Pyrethrins and in particular bioallethrin interact strongly with SHBG at a concentration of 40 mg/kg. The author's advice protection from any form of contact or ingestion of the pyrethroid in order to prevent any undesirable effects on the human reproductive system until additional toxicological and endocrine studies can be conducted [13].

This study was performed to evaluate the effect of deltamethrin (DLM) insecticide on male fertility in rats and to examine the possible *protective effect of vitamin E against reproductive toxicity of* DLM.

Material and Methods

Drug:

Deltamethrin (Butox® 50 EC) was obtained from Intervet Schering-Plough Animal Health Company) while Vitamin E (Alpha-tocopherol) was obtained from Pharco Company for pharmaceuticals, Alexandria, Egypt, in the form of soft gelatine capsules each containing 1000 mg of vitamin E.

Animals and Experimental Design

A total of 25 Sprague-Dawley sexually mature male rats weighing from 180 to 200 g b.wt and 10-12 weeks old were used in this study. Animals were reared at Pharmacology Department, Faculty of Veterinary Medicine, Giza, Egypt. Rats were kept under hygienic conditions, fed on rat pellets which composed of 10% wheat bran, 44% soy bean powder, 22% net protein, 4.7% fats, 3.3% fibres, fish meal, molasses, salts (sodium chloride, calcium carbonate, and calcium phosphate) and methionine. These pellets were manufactured by Cairo Agriculture Development Company, 6th October City, Giza, Egypt. Water was provided to rats *ad libitum*. The experiment on rats was carried out in accordance with the recommendations of the National regulations and rules on animal welfare and Institutional Animal Ethical Committee (IAEC). The animals were subdivided into 5 groups of sexually mature male rats, each of 5 animals. Group (1) was kept as control group negative (non-treated); groups (2) and (3) were orally given deltamethrin alone in a dose of 6 and 3 mg kg-1 b.wt. once daily for 65 consecutive days, respectively. Groups (4) and (5) were orally given deltamethrin in a dose of 6 and 3 mg kg-1 b.wt. in combination with vitamin E (40 mg/kg b.wt.) once daily for 65 consecutive days, respectively.

Blood Sampling and Hormonal Assay

Blood samples were collected from the veins of orbital plexus of each animal at the end of experiment. Serum was separated for estimation of testosterone, FSH and LH level. Testosterone concentration was assayed using radioimmunoassay (RIA) method as described [14] while LH and FSH concentrations were determined by ELISA technique [15].

Effect on Male Fertility

Sex Organs Weight

Five rats from each group were sacrificed at the end of experiment. The testes, prostate gland and seminal vesicles were dissected and weighed in relative to body weight.

Semen Analysis

Seminal contents of the epididymis were obtained after cutting the cuda epididymis and squeezing it on a clean sterile glass slide. The sperm progressive motility and sperm cell concentration were determined. The percentage of sperm cell viability and morphological abnormalities were assessed using an Eosin - Nigrosin stain [16].

Assessment of Testicular Antioxidant Enzymes

Testicular tissue specimens were homogenized in 9 fold volumes phosphate buffered solution (PH 7.4). The homogenate was then centrifuged at 4000 rpm for 15 min at 4 °C and the supernatant was kept at -80^{o}C until used. Superoxide dismutase (SOD) activity was determined as described by [17] while Glutathione peroxidase (GPx) and Catalase (CAT) activities were measured according to [18] and [19] respectively.

Histopathological Examination

Specimens from testis were collected from all experimental groups and fixed in 10% neutral buffered formalin, dehydrated in ascending concentrations of ethyl alcohol (70-100%). Specimens were prepared using standard procedures for Hematoxylin and Eosin staining as described by [20].

Statistical Analysis

Data were expressed as means ± standard errors (S.E.) and were statistically analyzed using one-way analysis of variance (ANOVA). That followed by Turkey-Kramer's multiple comparison tests at significant level $P<0.05$ [21].

Results

Oral administration of deltamethrin to male rats at a dose of 1/10 and 1/20 of LD_{50} for 65 consecutive days caused significant decreases in the relative weight of testes, epididymis, seminal vesicles and prostate gland. Concurrent administration of deltamethrin (DEM) with vitamin E to male rats resulted in non-significant increase in relative weights of these sex organs when compared to the effect of deltamethrin alone (Table 1).

Semen analysis revealed that oral administration of DEM caused significant decrease in sperm cell concentration and percentage of sperm motility and viability (Table 2). There was also significant increase in sperm cell abnormalities. Co-administration of vitamin E with DEM significantly increased sperm cell concentration with non-significant increase in percentage of sperm motility and viability associated with non-significant decrease in sperm cell abnormalities.

Table 1: Effect of deltamethrin alone and/or in combination with vitamin E on relative weights of sexual organs of male rats. (Means ± S.E., n=5)

Groups	Relative weight of sex organs (g/100 g b.wt.)			
	Testes	**Epididymis**	**Prostate glands**	**Seminal vesicles**
Negative control	0.502±0.016[a]	0.31±0.02[a]	0.41±0. 06[a]	0.472±0.01[a]
DEM (6 mg/kg)	0.36±0.014[b]	0.19±0.028[c]	0.27±0.04[c]	0.29±0.03[c]
DEM (3 mg/kg)	0.36±0.015[b]	0.23±0.018[b]	0.31±0.04[b]	0.36±0.07[b]
DEM (6 mg/kg) + Vit. E (40 mg/kg)	0.388±0.01[b]	0.22±0.018[b]	0.33±0.02[b]	0.34±0.04[b]
DEM (6 mg/kg) + Vit. E (40 mg/kg)	0.392±0.025[b]	0.22±0.019[b]	0.34±0.09[b]	0.36±0.04[b]

Means ±S.E with different superscripts in the same column are significant at P< 0.05 using one way ANOVA test.

Table 2: Effect of oral administration of deltamethrin alone and in combination with vitamin E on sperm parameters of male rats. (Means ± S.E., n=5)

Groups	Count (10^6/ml)	Motility (%)	Viability (%)	Abnormality (%)
Negative control	75.0±1.7[a]	91.6±1.9[a]	90.2 ±1.5[a]	7.6±1.2[d]
DEM (6 mg/kg)	45.0±1.4[c]	55.0±3.9[c]	50.0±2.5[c]	20.6±1.4[a]
DEM(3 mg/kg)	46.0±0.6[c]	62.0±2.0[c]	54.0±1.9[c]	16.0±1.1[b]
DEM(6 mg/kg) + Vit. E (40 mg/kg)	53.0±0.7[b]	68.0±2.1[b]	56.0±1.7[c]	14.8±1.3[c]
DEM(6 mg/kg) + Vit. E (40 mg/kg)	57.0±1.7[b]	71.0±0.8[b]	63.0±2.5[b]	13.4±0.4[c]

Means± S.E with different superscripts in the same column means significant difference at P< 0.05 using one way ANOVA test.

Assessment of antioxidant enzyme activities in testicular tissue of male rats revealed that DEM induced significant decrease in the activities of testicular superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT). Concurrent administration of vitamin E and deltamethrin resulted in significant increases in the activities of testicular superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes as shown in Table (3).

Table 3: Effect of oral administration of deltamethrin alone and in combination with vitamin E on anti-oxidant enzyme activities in testicular tissue of male rats (Means ± S.E., n=5)

Groups	SOD (U/mg protein)	GPx (nmol/min/mg protein)	CAT (nmol/min /mg protein)
Negative control	22.50±0.05[a]	254.5±5.6[a]	272.5±9.5 [a]
DEM (6 mg/kg)	16.25±0.06[c]	182.6±6.2[c]	155.6±9.2[c]
DEM(3 mg/kg)	17.45±0.04[c]	185.6±6.6[c]	153.2±8.5[c]
DEM+ (6 mg/kg) Vit. E (40 mg/kg)	20.25±0.04[b]	222.6±5.8[b]	217.2±7.3[b]
DEM +(3 mg/kg) Vit. E (40 mg/kg)	21.55±0.02[b]	223.6±6.8[b]	213.5±7.3[b]

Means± S.E with different superscripts in the same column means significant difference at P< 0.01 using one way ANOVA test.

GPx unit = nmol of GSH utilized/min/mg protein.

CAT unit = nmol of H_2O_2 utilized/min/mg protein.

There were significant decreases in serum testosterone (TH), FSH and LH hormones levels in male rats when orally given deltamethrin at dose of 1/10 and 1/20 LD50 for 65 consecutive days. Concurrent administration of vitamin E with deltamethrin (DEM) when given to male rats at a dose of 1/10 for 65 consecutive days resulted in significant increases in serum levels of TH, FSH and LH.

Table 4: Effect of oral administration of deltamethrin (DEM) alone and in combination with vitamin E (Vit. E) on serum testosterone, FSH and LH in male rats. (Means ± S.E., n=5)

Groups	Testosterone (mg/mL)	FSH (mg/mL)	LH (mg/mL)
Negative control	3.90±0.04[a]	2.20±0.037[a]	0.38±0.012[a]
DEM(6 mg/kg)	2.68±0.07[b]	1.72±0.058[b]	0.34±0.015[b]
DEM(3 mg/kg)	2.98±0.07[b]	1.80±0.044[b]	0.35±0.015[b]
DEM(6 mg/kg) + Vit. E (40 mg/kg)	3.10±0.04[a]	1.86±0.05[b]	0.35±0.012[b]
DEM (3 mg/kg) + Vit. E (40 mg/kg)	3.20±0.06[a]	1.90±0.031[a]	0.38± 0.013[a]

Means± S.E with different superscripts in the same column denotes Significant difference at P< 0.05 using one way ANOVA test.

Histopathological Examinations

Histopathological examination of the testes showed degeneration of seminiferous tubules and edema in the interstitial tissue in the group given deltamethrin at 1/10 and at 1/20 of LD_{50} as demonstrated in Fig. (1) and Fig. (2). The degenerated seminiferous tubules exhibited few layers of spermatogenic cells and few sperms in the lumen. The testes of rats given deltamethrin at 1/10 of LD_{50} and vitamin E exhibited seminiferous tubules that were lined with spermatogenic cells up to sperm formation in the lumen (Fig. 3). Rats administered with deltamethrin at1/20 of LD_{50} and vitamin E revealed a better sperm formation in the lumen of the seminiferous tubules (Fig. 4).

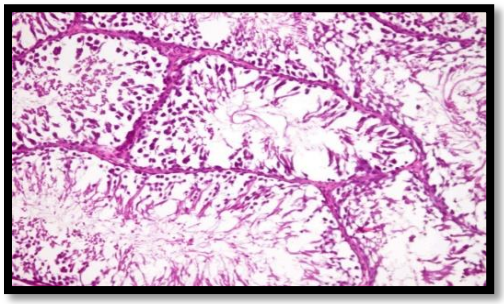

Figure 1: Testis of rat treated with deltamethrin 1/10 of LD_{50} showing severe interstitial edema, (H&E X 200).

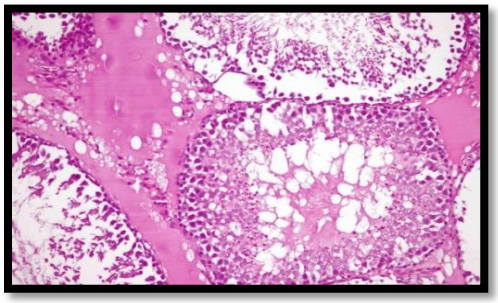

Figure 2: Testis of a rat treated with deltamethrin 1/20 of LD_{50} showing few layers of spermatogonial lining seminiferous tubules (H&E X 200).

Figure 3: Testis of rat treated with deltamethrin 1/10 of LD_{50} and vitamin E showing seminiferous tubules lined by spermatogonial cells (H&E X 100).

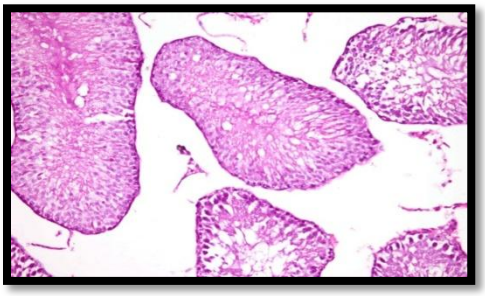

Figure 4: Testis of rat treated with deltamethrin 1/20 of LD_{50} and vitamin E showing seminiferous tubules lined by spermatogonial cells up to sperm formation (H&E X 200)

Discussion

In this study, oral administration of deltamethrin (DLM) alone at doses 6 and 3 mg/kg for 56 days to rats reduced male fertility, decreased sexual organs weight, sperm concentration, motility and viability; but increased percent of spermatozoa abnormalities associated with decreased sex hormone serum levels. These findings were similar to those are already reported in rats and mice [22-29].

Concurrent administration of deltamethrin with vitamin E to male rats significantly evoked protective and antioxidant activities against toxic effect of deltamethrin. These protective effects were evident by significant increases in the relative weight of the testes, increases in serum levels of testosterone and FSH hormones, improvement of semen quality and quantity and alleviation the testicular degenerative lesions seen in the testes. The activity of antioxidant SOD and CAT enzymes was also increased in the testicular tissue. The protective and antioxidant activities of vitamin E against toxic effect of deltamethrin were in accordance with the previous findings of [11]. The previous authors concluded that vitamin E can protect sperm DNA from oxidative stress in the rat testis and enhance spermatogenesis and male fertility due to its powerful antioxidant activity. Moreover, combination of vitamin E and selenium improved semen parameters in infertile men as reported by [30]. In the current study, the results concerning vitamin E were nearly similar to previous reports that vitamin E increased sperm count, motility and viability and increased serum testosterone, FSH, and LH levels. There were also alleviation of testicular degenerative changes including degeneration, congestion, and edema induced by lead acetate in vitamin E - administered rats.

The increase of serum sex (testosterone, LH and FSH) hormones levels caused by co-administration of vitamin E with deltamethrin, in this study, might be responsible for improving semen quality and quantity. It has been established that testosterone is essential for spermatogenesis, and also FSH plays a valuable role in germ cell progression and improves fertility in animal models [31]. Moreover, it well known that the testicular function and spermatogenesis are controlled by FSH- and LH-linked mechanisms [15]. Administration of vitamin E with deltamethrin in combination induced antioxidant effect in the testes of rats. This effect was evident from the increased activities of testicular superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) antioxidant enzymes. The reported antioxidant affect of vitamin E agreed with the previous findings [11,32-33].

Conclusion

In conclusion, oral administration of deltamethrin at doses of 6 and 3 mg/kg b.wt for 56 days to male rats causes reproductive toxicity. This toxicity is manifested by decreased weights of testes, lowered semen quality and

quantity, decreased serum sex hormone levels and decreased activity of testicular antioxidant enzymes. Coadministration of vitamin E with deltamethrin induces beneficial protective and antioxidant effects against reproductive toxicity induced by deltamethrin in male rats.

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