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Protective effect of vitamin E and selenium combination on deltamethrin-induced reproductive toxicity in male rats

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ABSTRACT

The current study was performed to assess the adverse effect of deltamethrin (DLM) on reproductive organs and fertility in male rats and to evaluate the protective role of vitamin E (VE) and selenium (Se) combination in alleviating the detrimental effect of DLM on male fertility. The lethal dose 50 (LD_{50}) of DLM for male rats was estimated at 6 mg/kg bwt. Thirty male albino rats (10-weeks-old) were divided into three groups (10 rats each): Control group was injected subcutaneously with 2 ml/kg bwt saline twice weekly and was daily administered 2 ml distilled water intra-gastrically; DLM-treated group received 0.6 mg/kg bwt (1/10 LD_{50}) DLM intra-gastrically once daily; DLM + VE/Se-treated group was injected subcutaneously with 1.2 mg/kg bwt Viteselen® 15 (VE/Se) twice weekly with concurrent daily administration of 0.6 mg/kg bwt (1/10 LD_{50}) DLM intra-gastrically. The experiment was conducted for 60 consecutive days. DLM caused a significant reduction in reproductive organs weights, sperm count, sperm motility percent, alive sperm percent, serum testosterone level and testicular reduced glutathione concentration (GSH). DLM-treated group showed a significant increase in sperm abnormalities and testicular malondialdehyde (MDA) concentrations. Histopathologically, DLM caused impairments in testes, epididymes and accessory sex glands. Conversely, treatment with VE/Se combination improved the reduction in the reproductive organs weights, sperm characteristics, DLM-induced oxidative damage of testes and the histopathological alterations of reproductive organs. Results indicate that DLM exerts significant harmful effects on male reproductive system and that the concurrent administration of VE/Se partly reduced the detrimental effects of DLM on male fertility.

1. Introduction

Synthetic pyrethroids are modified derivatives of pyrethins, natural substances obtained from flowers of *pyrethrum* species (Luty et al., 2000). Concerning to their high bioefficacy at low concentrations, enhanced photostability and relatively low mammalian and avian toxicity, pyrethroid insecticides are widely used in agriculture, domestic and veterinary applications than other insecticides, particularly organochlorine, organophosphate and carbamate insecticides (Pauluhn, 1999).

Deltamethrin (DLM), a synthetic pyrethroid type II, is commonly used in Egypt for agriculture, veterinary and public health applications. In veterinary practice, it is used for the control of ectoparasites in domesticated animals and poultry. The most important sources of the animal and human exposure to DLM are polluted food and water, and it is readily absorbed by the oral route (Barlow et al., 2001). DLM has a deleterious effect of on male fertility (Abd El-Aziz et al., 1994; El-Gohary et al., 1999). During pyrethroid metabolism, reactive oxygen species (ROS) are generated and result in oxidative stress in intoxicated animals (Kale et al., 1999). In mammals, sperm plasma membranes have extremely high concentration of polyunsaturated fatty acids and insufficient antioxidant defenses; hence they are highly susceptible to lipid peroxidation (Aitken et al., 1993). The production of ROS is a normal physiological event in various organs including the testis controlling sperm capacitation, acrosome reaction and sperm–oocyte fusion. However, overproduction of ROS can be harmful to sperm and subsequently to male fertility (Akiyama, 1999).

The natural antioxidants may be helpful in preventing or reducing the harmful effects of ROS on testes and semen quality (Yousef, 2010). Vitamin E (VE) is the main component of the antioxidant system of the spermatozoa and is one of the major membrane protectants against ROS and lipid peroxidation (Akiyama, 1999). Supplemental VE increases total sperm output and sperm concentration in rabbits (Yousef, 2010) and rams (Yue et al., 2010). Conversely, deficiency of VE may lead to detrimental effects on the reproductive organs, such as degenerative spermatogonium, testicular damage and degeneration of the seminiferous tubules

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(Wilson et al., 2003). The protective effect of VE on pesticide induced oxidative stress has been reported (Yousef, 2010). Selenium (Se), a potent antioxidant, constitutes an essential component of glutathione peroxidase (GSH-Px) and selenoprotein P (Rotruck et al., 1973), those have ROS-scavenging activities and can protect membrane lipids and macromolecules against oxidative damage. Testes and epididymes contain high concentrations of Se indicating its vital role during spermatogenesis to improve semen quality (Ranawat and Bansal, 2009). It has been found that Se deficiency may lead to various reproductive disorders, e.g. seminiferous tubule degeneration, poor spermatozoa integrity, reduced numbers of spermatozoa within the seminiferous tubules, and reduced sperm motility (Marin-Guzman et al., 2000). Influence of dietary Se on semen quality has been described in mice (Sánchez-Gutiérrez et al., 2008), rat (Wu et al., 1979), rabbit (Cesare et al., 2002) and goats (Shi et al., 2010). The concurrent use of VE and Se has a synergistic protective effect against oxidative damage (Cemek et al., in press). Consequently, this study aimed to evaluate (1) the influence of DLM on reproductive organs and fertility of male albino rats, (2) the protective role of VE and Se in alleviating the detrimental effect of DLM on male fertility.

2. Materials and methods

2.1. Chemicals

Commercially grade deltamethrin-based pesticide (Butox® 5%EC) (Intervet Co., France) was used in this study. Viteselen®15 (contains 1.67 mg sodium selenite plus 150 mg Vit E/ml) was obtained from Adwia Co. S.A.E.

2.2. Animals and experimental design

Sixty adult male Wister rats (weighing 150–170 g, 10 weeks age) were obtained from a closed random bred colony at the Medical Research Institute of Alexandria University, Egypt. Animals were housed in plastic cages with free access to the commercial basal food and water. The standard laboratory diet was purchased from Damanhur Feed Co. (Behera, Egypt). The animals were acclimatized 2 weeks prior to the experiments. Rats were received humane care in compliance with the guidelines of the National Institutes of Health (NIH) of Animal Care and the local committee approved this study.

2.2.1. Estimation of lethal dose$_{50}$ (LD_{50}) of deltamethrin

Twenty-four rats were orally administered deltamethrin with different four concentrations (1, 3, 5, 7 mg/kg bwt). Six rats were kept as control group throughout the entire experimental period. Mortality was assessed and counted in the different groups. LD_{50} was calculated according to Behrens and Karber (1953).

2.2.2. Evaluation of the protective effect of Viteselen®15 on deltamethrin-induced-oxidative damage on male fertility

Thirty adult male Wister rats were divided into three groups (10 rats each): Group A (Control rats) was injected subcutaneously with 2 ml/kg bwt saline (vehicle of Viteselen®15) twice weekly and was daily administered 2 ml distilled water (vehicle of deltamethrin) intra-gastrically using stomach tube. Group B (DLM-treated rats) received deltamethrin (0.6 mg/kg bwt equals to 1/10 LD_{50}) intra-gastrically once daily. Group C (DLM+VE/Se-treated rats) was injected subcutaneously with 1.2 mg/kg bwt Viteselen®15 (Paget and Barnes, 1964) twice weekly with concurrent daily administration of deltamethrin (0.6 mg/kg bwt equals to 1/10 LD_{50}) intra-gastrically. The experiment was conducted for 60 consecutive days taking into consideration the period necessary to complete a spermatogenic cycle (Clermont and Harvey, 1965).

2.3. Reproductive organs weights

All rats were euthanized at the end of the experiment. After animal dissection, the testes, epididymes and accessory sex organs (seminal vesicles and prostate glands) were removed, grossly examined and weighed. The index weight (I.W.) of each organ was calculated by Matousek (1969) I.W. = organ weight (g)/100 × body weight (g).

2.4. Epididymal sperm count

Epididymal spermatozoa were counted by a modified method of Yokoi et al. (2003). Briefly, the epididymis was minced in 5 ml of saline, placed in a rocker for 10 min and incubated at room temperature for 2 min. The supernatant fluid was diluted 1:100 with a solution containing 5 g $NaHCO_3$, 1 ml formalin (35%) and 25 mg eosin per 100 ml distilled water. About 10 μl of the diluted semen was transferred to each counting chamber of the improved Neubaur haemocytometer (Deep 1/10 mm, LABART, Munich, Germany) and was allowed to stand for 5 min for counting under a light microscope at ×200 magnification.

2.5. Epididymal alive sperm percent

A drop of epididymal contents of each rat was mixed with an equal drop of eosin–nigrosin stain. The semen was carefully mixed with the stain and thin film was spread on a clean slide. Two hundred sperms were randomly examined per slide at ×400 magnification according to Bearden et al. (1980).

2.6. Sperm motility

Sperm-progressive motility was evaluated microscopically within 2–4 min of their isolation from the cauda epididymis as described by Sönmez et al. (2005). Fluid was obtained from the cauda epididymis with a pipette and diluted to 2 ml with tris buffer solution. The percentage of motility was evaluated at ×400 magnification.

2.7. Sperm abnormalities

A total of 300 sperm was counted on each slide under light microscope at ×400 magnification and the percentages of morphologically abnormal spermatozoa (detached head and coiled tail) were recorded according to Evans and Maxwell (1987).

2.8. Determination of serum testosterone levels

Blood was collected from retro-orbital plexus of all anesthetized rats before scarification. Serum was separated for assessment of the total serum testosterone according to Demetrious (1987) using solid phase radioimmunoassay (RIA) kits. This assay based on testosterone-specific antibody immobilized to the wall of a poly propylene tube.

2.9. Antioxidant enzyme activity and oxidative stress assays

One testis of each rat was kept frozen at −70 °C for assessment of reduced glutathione (GSH) activity and lipid peroxidation (LPO) content. GSH activity was assessed spectrophotometrically according to Sedlack and Lindsay (1968). The method is based on the reductive cleavage of 5,5-dithiobis-(2-nitrobenzoic acid) by sulfhydryl (–SH) group to yield a yellow colour with maximum