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Epithelial and stromal alterations in prostate after cypermethrin administration in adult albino rats (histological and biochemical study)

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ABSTRACT

Histological and biochemical alterations induced in prostate by cypermethrin insecticide exposure were investigated in adult albino rats. 60 mg/kg/day of cypermethrin were given orally to experimental group for 15 days then prostatic specimens were processed for light and electron microscopic examinations and for assessment of oxidative stress markers; prostatic glutathione (GSH), glutathione peroxidase enzyme (GPx) and malondialdehyde (MDA). Masson's trichrome and anti-α-actin antibodies immunohistochemical staining were done. Blood samples were collected for measurement of total and prostatic acid phosphatase enzymes. Morphometric and statistical analyses were conducted. Cypermethrin treated group showed decrease in acinar epithelial height with detection of heterochromatic nuclei, cytoplasmic vacuolations and few apical microvilli. The stroma surrounding the acini was widened with significant increase in collagen fibers and significant decrease in smooth muscle cell α-actin immunoexpression. This was accompanied by a significant decrease of prostatic GSH level, activity of GPx enzyme with a significant increase in MDA level. Significant decrease in total and prostatic enzyme activities was also detected. In conclusion, cypermethrin induced epithelial degenerative changes in prostate which were accompanied by stromal alterations that seemed to be due to oxidative stress. More attention is required to the role of stromal microenvironment and oxidative stress markers in prostatic diseases.

1. Introduction

The undesired effects of chemical pesticides was recognized as a serious public health concern during the past decades (Giri et al., 2003). Pyrethroid pesticides are a group of man-made products which use was increasing during the past decade with the declining use of organophosphate pesticides, which are more acutely toxic to birds and mammals than pyrethroids. Cypermethrin is a very active synthetic pyrethroid insecticide which is widely used in and around households as well as in agriculture (Shafer and Meyer, 2004; Solati et al., 2010). Cypermethrin was considered to be safe for mammals however, it was suggested that it can induce tissue damage through free radical formation and reduced antioxidant defense mechanism leading to neurotoxicity in rats (Kamel, 2011; Sharma et al., 2014) and reproductive toxicity in mice (Wang et al., 2009). It was shown that some pyrethroids pesticides are associated with certain male reproductive damages including reduced sperm count, testicular lesions, sperm motility changes, sperm morphologic abnormality and genotoxic effects (Bian et al., 2004; Song et al., 2008). Cypermethrin have been classified as endocrine-disrupting compounds as they possess hormonal activities which potentially pose a threat to human and wildlife (Kim et al., 2005; Han et al., 2008). This hormonal activity was reported to be through estrogenic activities of pyrethroids (Wilson et al., 2004; Kojima et al., 2004). Hence, much attention has been recently paid to anti-androgenic effects of the pesticides for the observed degeneration in male reproductive health (Bian et al., 2004; Song et al., 2008).

The prostate gland is the largest of the male accessory which contracts with ejaculation providing enzymes to maintain the fluid nature of seminal fluid and to nourish sperm as they pass to outside the body. It is a complex tubulo-alveolar gland composed of an epithelial parenchyma embedded within a stromal tissue (Cunha et al., 2004). In normal rodent and human prostates, fibroblasts and smooth muscle cells (SMCs) predominate in the stromal compartments. SMCs of prostate stroma was reported to express α-actin whereas fibroblasts express vimentin (Berry et al., 2008).

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Stromal fibromuscular compartment was reported to modulate epithelium growth and maintain cellular homeostasis through identified growth factors (Lai et al., 2012). However, modified stromal cells was reported to secrete extracellular matrix proteins and soluble factors, which in turn play important roles in carcinoma development (Niu and Xia, 2009). The understanding of the prostatic microenvironment is the subject of intense therapeutic interest as its phenotypic and molecular characterization has been correlated with prostatic diseases (Ayala et al., 2003; Finak et al., 2008; Saadi et al., 2010).

Acid phosphatase, an enzyme of a lysosomal origin, is detectable in all germinal cells and its specific activity increases with the development of spermatocytes. Activities of free lysosomal enzymes have been shown to rise when testicular steroidogenesis is increased (Chitra et al., 1999). Reactive oxygen species have an important role in the pathogenesis of infertility. Oxidative stress developed when there is an imbalance between the generation of reactive oxygen species (ROS) and the scavenging capacity of the antioxidants such as glutathione (GSH) and glutathione peroxidase (GPx) in the reproductive tract (El-Tohamy and El-Nattat, 2010). Hence, the objective of this study was to elucidate toxic effects of cypermethrin on prostatic function and structure using light electron microscope and biochemical methods with special emphasis on molecular features of the stromal microenvironment and the role of antioxidants disruption in these toxic effects.

2. Materials and methods

2.1. Materials

2.1.1. Chemicals

Cypermethrin (a-cyano-3-phenoxy-benzyl 3-(2,2-dichlorovinyl) 2,2-dimethylcyclopropane-carboxylate): a commercial emulsifiable concentrate was supplied by the Central Agricultural Pesticides Laboratory, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt. Kits for estimation of total and prostatic acid phosphatase provided by Quimica Clinica Aplicada S.A., Tarragona, Spain. Kits for estimation of glutathione (GSH), glutathione peroxidase (GPx) and malondialdehyde (MDA) levels provided by Bio-diagnostic Co., 29 El-Tahrer Street, Dokki, Giza, Egypt.

2.1.2. Experimental animals

Forty adult male albino rats (6 months old and 240 ± 20 g) were obtained from the Egyptian Organization for Biological Products and Vaccines. Rats were kept in individual polyethylene cages with stainless-steel tops at the Animal House, Faculty of Medicine, Zagazig University. Rats were subjected to controlled conditions of temperature (25 ± 2 °C) and illumination (12 h-light/dark), and allowed free access to normal rat chow diet and water ad libitum. The experiment was carried out in compliance with the "Guide of the Care and Use of Laboratory Animals" (Institute of Laboratory Animals Resources, 1996). Experimental protocols were approved by the ethical committee of the Faculty of Medicine, Zagazig University.

One week after acclimatization rats were randomly divided into two major equal groups:

Group I *(control group)*: Included twenty rats, divided into 2 equal subgroups, 10 rats each:

Subgroup Ia (Negative control group): Each rat of this group received regular diet and water for 15 consecutive days.

Subgroup Ib (Positive control group): Each rat of this group received regular diet, water and corn oil (vehicle of cypermethrin) in a dose of 1 ml/kg/day for 15 consecutive days.

Group II *(Cypermethrin group)*: Included twenty rats. Each rat received 60 mg/kg/day cypermethrin dissolved in 1 ml of corn oil by orogastric tube for 15 consecutive days (Fang et al., 2013).

2.2. Methods

2.2.1. Histological and immunohistochemical study

In the day 15 after treatment, rats were anaesthetized using ether inhalation, 2 h following the last dose, the ventral prostatic lobes were dissected out and samples from intermediate and distal regions of the ventral prostatic lobes were taken (Favaro et al., 2009) and divided into three parts; one was processed for light microscopy, for electron microscopy and part for biochemical study. For light microscope, the specimens were fixed in Bouin's solution for 12 h to prepare paraffin blocks. Thick sections (5 μm) were prepared and stained by hematoxylin and eosin stain (H&E) stain and Masson's trichrome stain for demonstration of collagen fibers (Bancroft and Gamble, 2008). For immunohistochemical study, the deparaffinized sections on charged slides were used for localization of SMCs α-actin protein using avidin–biotin-complex (ABC) immunoperoxidase technique. The sections were incubated in hydrogen peroxide for 10 min to block the endogenous peroxidase then incubated with the primary anti-α-actin antibody at 1:5000 dilutions for 30 min at room temperature. The primary antibody used was a mouse monoclonal antibody with cytoplasmic reaction specific for SMCs. α-Actin protein obtained from Lab Vision Corporation (Cat. #MS-113-PO). Then the slides were washed with phosphate buffer then incubated with the secondary anti-mouse antibodies universal kits obtained from Zymed Corporation. Staining was completed by incubation with substrate chromogen DAB (3,3′-diaminobenzidine) which resulted in brown-colored precipitate at the antigen sites and Mayer's hematoxylin was used as a counter stain. Positive control was smooth muscle cells of small intestine. Negative control sections were prepared using PBS without using the primary antibody (Kiernan, 1999).

2.2.2. Ultrastructure study

For transmission electron microscope, 1 mm^3 specimens were immediately fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at pH 7.4 for 2 h at 4 °C. Then the specimens were washed with the buffer, post fixed in 1% osmium tetroxide in distilled water for 2 h at 4 °C. Specimens were dehydrated with ascending grades of ethanol and then put in propylene oxide to prepare Epon-Araldit resin blocks Semithin sections (0.5 μm) were stained by toluidine blue (Glauret and Lewis, 1998). Ultrathin sections (70–90 nm) were obtained, stained by uranyl acetate and lead citrate and examined under JEOL 1010 electron microscope, in Histology Department, Faculty of Medicine, Zagazig University.

2.2.3. Morphometric study

The image analyzer computer system Leica Qwin 500 in the Histology Department, Faculty of Medicine, Zagazig University was used to evaluate the following parameters in 10 consecutive fields from each rat in randomly chosen five animals of each group.

2.2.3.1. Using H&E stained sections. The epithelial height of acini was measured, using the interactive measure menu. These measurements were taken using total magnification ×400.

2.2.3.2. Using Masson's trichrome-stained sections. The mean area percentage of the collagen content in the stroma between the acini, ducts, and around blood vessels were measured using the interactive measure menu using total magnification ×100. The area percentage and standard measuring frame of a standard area equal to 118,476.6 mm^2 were chosen from the parameters. In each randomly chosen field, the section of the prostate was enclosed inside