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Chlorpyrifos induced toxicity in reproductive organs of female Wistar rats

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ABSTRACT

Chlorpyrifos (CPF) is an organophosphate (OP) insecticide extensively used in agricultural and domestic settings. Healthy adult female albino rats were divided into three groups of six rats in each. Two groups were dosed orally with CPF in vegetable oil (0.1 and 2.5 mg/kg/day) and third group was given vegetable oil for 8 weeks. Non-significant changes were observed for body weight and feed intake. A disruption in estrous cyclicity was observed with a prolonged metestrous. Erythrocyte osmotic fragility and lipid peroxidation levels increased significantly. Mammary gland whole mounts revealed a significant ($P < 0.05$–0.0001) increase in the ductal thickness, number of branches, alveolar and terminal end bud number and terminal end bud diameter. A significant increase in ovarian surface epithelium height, follicular diameter and follicular atresia was observed in treated rats ($P < 0.05$–0.0001). A similar significant increase in the uterine surface epithelium height, endometrial gland epithelium height and myometrium thickness in higher dose group was recorded ($P < 0.05$–0.0001). Luminal epithelium height and endometrial gland diameter was increased significantly in both the treated groups ($P < 0.05$–0.0001). The results indicate that sub-chronic exposure of CPF causes oxidative stress and negative effects on the reproductive organs of female rats, which may be a pointer towards beginning of cancer incidence.

1. Introduction

Organophosphate insecticides represent a major class of agricultural chemicals that are used to control a wide range of sucking and chewing pests of field crops, fruits and vegetables. Blood disorders (anaemia, defective blood coagulation), brain and nerve damage, paralysis, jaundice, hepatic fibrosis, allergenic sensitization, emphysema, asthma, kidney problems, cancer, genetic disorders, birth defects, miscarriage, impotence, and infertility or sterility are variously associated with chronic exposure and low residual level of pesticides (Gold et al., 2001).

Chlorpyrifos (CPF) is one such broad spectrum organophosphate (OP) insecticide used for a variety of agricultural and domestic applications (EPA, 2008). It is the fourth highest consumed insecticide in India and residues have been detected at a mean level of 0.0662 mg/L in human blood, 1.45 mg/kg in eggplant, 0.092 μg/mL in milk samples from cow, buffalo, doe and ewe (Mathur et al., 2005; Baig et al., 2009; Karabasanavar and Singh, 2012). A decreased body weight, appetite, milk production and interrupted estrous cycle have been found to be associated with continuous low-level exposure to pesticides in human, laboratory and farm animals (Tiemann, 2008). Reproductive behaviour is considered as a promising tool in ecotoxicology which provides information about the biochemical, physiological and toxicological reactions to a chemical related to reproduction (El-Kashoury and El-Din, 2010; El-Mazoudy et al., 2011; Attia et al., 2012) and many of the tested pesticides have been found to be more toxic to females than males of a species (Mancini et al., 2005). Chlorpyrifos has been found to cause some developmental defects when exposed orally in rats (Adigun et al., 2010; Shalaby et al., 2013). Insecticides have been found to disturb biochemical and physiological functions of red blood cells (RBC) through lipid peroxidation (Akhgari et al., 2003; Ambali et al., 2010). An increased lipid peroxidation and decreased level of follicle stimulating hormone (FSH) in CPF treated albino mice has also been observed by Madhavi and Kumar (2010). The present laboratory based study was carried out to study the CPF-induced erythrocyte lipid peroxidative and histological changes in reproductive organs of female Wistar rats to bring forth the changes at the cellular level in individuals chronically exposed to the pesticide.

2. Materials and methods

2.1. Experimental animals

Healthy adult female Wistar albino rats of 12 weeks of age and weighing between 140 and 160 g were procured from Department of Livestock Production and Management, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India. Animals were maintained at the Animal House in the

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Department of Zoology, Punjab Agricultural University, Ludhiana in polypropylene cages under controlled conditions (23 ± 2 °C temperature; 40 ± 5% relative humidity). Water and standard pelleted feed were provided *ad libitum*. Rats were acclimatized to the laboratory environment for 15 days prior to the start of experiments. The Institutional Animal Ethics Committee (IAEC) approved this experimental protocol (VPS/2008/874-885).

2.2. Chemicals and experimental designs

Commercial grade Chlorpyrifos (Eldrin, 20EC) was purchased from Crystal Phosphate Limited, Nathupur, Sonepat, Haryana, India. Different dilutions for the doses of the insecticide to be administered were made with vegetable oil. The lower dose was selected on the basis of no observed effect level (NOEL, 0.1 mg/kg bw) as determined in previous studies (Breslin et al., 1996; Marty et al., 2012) and the higher dose selected was half of the dose (5 mg/kg bw) used in a previous study which resulted in ovarian toxicity (Güney et al., 2007). The study was conducted for 8 weeks to determine intermediate toxic effects of CPF on female reproductive system.

The rats were divided into three groups of six animals each. Two groups were given Chlorpyrifos at a dose level of 0.1 mg/kg bw (T1) and 2.5 mg/kg bw (T2) for eight weeks on daily basis by oral intubation. Same amount of vegetable oil i.e. 1.25 ml/kg was given to the control group (C) orally through intubation. The animals were sacrificed on the completion of the experiment and the reproductive organs were collected, weighed and processed for analysis.

2.3. Body weight, feed intake and cyclicity

Rats were weighed before the start of the experiment and then weekly till the completion of the experiment. Daily feed intake was calculated during the experiment; the rats were also observed for any disturbance in the estrous cycle.

2.4. Evaluation of erythrocyte osmotic fragility

Blood samples were collected from the heart in heparinized vials and tested for erythrocyte osmotic fragility by the methods of Faulkner and King (1970) as modified by Oyewale (1992) using SL 159 UV–Vis spectrophotometer (Elico, Hyderabad, India) at a wavelength of 540 nm. The percent haemolysis for each sample was then calculated by the formula:

$$\%\text{Haemolysis} = \frac{\text{Optical density of the test solution}}{\text{Optical density of the standard}} \times 100$$

2.5. Evaluation of erythrocyte malondialdehyde concentration

Malondialdehyde (MDA) as a marker of lipid peroxidation was determined by the method of Draper and Hadley (1990) as modified by Altuntas et al. (2000). Heparinized blood samples (2.5 ml) were obtained from each animal, centrifuged by using cooling centrifuge C-30BL (REMI, Mumbai, India) at 3000 g at 4 °C for 5 min and the plasma was discarded. Separated erythrocytes were washed with cold isotonic saline (0.9% w/v) and used to analyze malondialdehyde (MDA) using SL 159 UV–Vis spectrophotometer (Elico, Hyderabad, India) at a wavelength of 532 nm. The concentration of MDA was calculated by the absorbance coefficient of MDA-TBA complex, $1.56 \times 10^5\ cm^{-1}\ M^{-1}$ and expressed in μmoles per gram of haemoglobin.

2.6. Histological studies

2.6.1. Mammary gland whole mount preparation

The animals were sacrificed after the completion of the experiment. The mammary glands were separated from the rats. Whole mounts were prepared by the method of de Assis et al. (2010) to be observed under light microscope (Olympus CH20i attached with Magnüs Micro Image Projection System, New Delhi, India). One whole mount per rat was prepared and observed with 10 fields of observation per whole mount.

2.6.2. Histology and micrometrical analysis

For histological and morphometrical analysis the ovaries and uteri were quickly removed and processed. The haematoxylin–eosin counter stained sections of ovary and uterus were analyzed using oculometer and stage-micrometer. For ovarian surface epithelium height 30 sections per ovary with 4 fields of observation per section were analyzed. In each sample serial sections were made and every 10th section was used to estimate the follicle number and size. The follicles having visible nuclei were counted. Approximately 30 sections per ovary were analyzed. For micrometrical analyses of uterus 10 sections per rat were used with 4 fields of observation per section. All histological evaluations were done without the knowledge of treatment.

2.7. Statistical analysis

Values obtained as mean ± SEM were subjected to one-way analysis of variance (ANOVA) followed by paired *t*-test, using GraphPad Prism version 6 from GraphPad Software, San Diego, CA, USA (www.graphpad.com). All the parameters were compared at 5% level of significance.

3. Results

3.1. Body weight, feed intake and cyclicity

No statistically significant change in the final body weight, net body weight gain, ovarian weight and feed intake was observed but a significant change in estrous cyclicity (Fig. 1A) and decreased growth rate was observed ($P < 0.05$) in lower dose group (0.802 ± 0.245 g/week/100 g bw) as compared to control (2.088 ± 0.613 g/week/100 g bw). The growth rate of higher dose group was comparable to control. The animals were in a prolonged state of metestrous during the treatment period in both the treated groups and this prolonged the 4-day cycling period to 5–7 day cyclicity.

3.2. Evaluation of erythrocyte osmotic fragility

A complete haemolysis (100%) in control solvent containing 0.0% NaCl concentration was observed and there was no significant difference in percent haemolysis at concentration of 0.1, 0.3, 0.5 g/L of NaCl in between control and treated groups. A significant difference ($P < 0.05$) was, however, observed at a higher concentration of 0.7 and 0.9 g/L. There was a significantly increased ($P < 0.05$) erythrocytic osmotic fragility at concentration of 0.5, 0.7 and 0.9 g/L in group T2 as compared to control (Fig. 1B) suggesting an onset of oxidative stress in the cells.

3.3. Erythrocyte malondialdehyde concentration

The effect of different treatments on erythrocyte MDA concentration depicted in Fig. 1(C) indicates a significant increase in the MDA concentration ($P < 0.05$) in both the treated groups as compared to control group.

3.4. Mammary gland

Micrometrical evaluation of mammary gland whole mounts revealed that there was a significant dose related increase in the interlobular ductal thickness in both (T1 and T2) CPF treated groups (Fig. 2A–C). Similarly, a statistically significant increase in the number of branches, terminal end buds (TEBs), alveolar buds (ABs) and terminal end bud diameter was observed (Table 1).

3.5. Ovary and uterus

The micrometrical analyses (Table 2) showed hypertrophy in the ovarian surface epithelium (OSE) and there was a statistically significant increase in OSE height in both the treated groups (Fig. 3A–C). A significant increase in the primordial follicle diameter in both the treated groups was recorded. The primary follicular diameter in T1 group was higher than control. The diameter of pre-antral follicles was higher in T2 group as compared to control. Similarly a significant increase in the antral follicular diameter was observed in both the treated groups. Follicular atresia was increased in the treated groups (Table 3, Fig. 4A and B).

Uterine absolute weight was found to be increased significantly in both the treated groups (Table 4). Micrometrical analyses of uterine tissues (Table 4) showed a significant increase in the uterine surface epithelium and luminal epithelium height in T2 group