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Effect of cypermethrin on the ovarian activity and its impact on fertility and pubertal onset of offspring

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ABSTRACT

Oral (by gavage) administration of cypermethrin (CYP), (3 doses; 1.38, 2.76 and 5.52 mg/kg body weight) to female mice for two durations (6 weeks and 12 weeks), caused significant reduction in the number of estrous cycles per month, serum levels of estradiol, total number of healthy ovarian follicles and number of corpora lutea whereas there was an increase in number of atretic follicles of all categories compared to controls. Mice of all treated groups after 6 weeks treatment showed 100% fertility. It was 80% in low and medium doses and 60% in high dose treatment after 12 weeks. There was a significant delay in the onset of puberty and reduction in number of estrous cycles of progeny of medium and high dose CYP treated mice mated with normal males. Number of estrous cycles per month, healthy follicles, corpora lutea and serum levels of estradiol, were not reversible within 6 weeks after cessation of medium and high dose treatment for 12 weeks. The study first time reveals that reversibility of the reproductive toxic effects of CYP depends on quantum and duration of exposure.

1. Introduction

Pesticides may enter the body of humans and domestic animals through various routes when these are introduced in the environment. Many studies reveal that exposure to pesticides affects the reproduction by interfering with the neurotransmission and endocrine regulation leading to alterations in the physiology of reproductive organs (Ahmad et al., 2009; Gill et al., 2011).

Although a substantial amount of research has been conducted to associate occupational exposure to pesticides with fertility problems in men (Wyrobek et al., 1981; de Cock et al., 1994), studies among women are scarce. Recently, an increased risk of infertility was observed among women exposed to pesticides or working in industries associated with agriculture (Fuortes et al., 1997). Infertility due to follicular dysfunction may be permanent or temporary. Hirshfield (1997) found that if primordial follicles were destroyed by a toxicant, it could lead to permanent infertility because of the eventual depletion of this large, non renewable pool of follicles. On the other hand, toxicant that damages only the primary or preantral follicles may result in temporary infertility, as when the exposure is leased primordial follicles, which are not affected can be recruited for growth to restore fertility. In women, there may be premature ovarian failure and early menopause if primordial follicles are destroyed extensively, as they are not renewed (Hoyer and Sipes, 1996; Hirshfield, 1997). Hence, there is a dire need to investigate pesticide induced changes in an ovarian follicular compliment and its impact on fertility.

There are a few studies on the effect of cypermethrin (CYP) a widely used pyrethroid on female reproduction in mammals. Administration of low doses of CYP up to 500 mg/kg body weight (bw) had no adverse effects on body weight and some reproductive parameters in mice, rats and rabbits (WHO, 1989). However, in a multigeneration study in rats, treatment with CYP at a dose of 500mg/kg in diet caused reduction in litter size and total litter weight (WHO, 1989). Some studies showed that CYP treatment caused embryonic resorption, pre and post implantation losses, decrease in number of implantation sites, number of viable fetuses and weight gain in fetuse in mammals (Rustamov and Abbasov, 1994; Biernacki et al., 1995; Elbetieha et al., 2001; Shukla and Taneja, 2002; Ullah et al., 2006). Administration of cypermethrin (10 mg/kg/day for 4 weeks, 5 days in a week) to adult female mice increased the number of dead pups in treated female rats mated with treated males (Farag et al., 2007). Daily administration with 100 mg CYP/kg bw in pregnant female rats from 6th to 17th day of gestation caused marked adverse effects in females and fetuses (Joshi et al., 2011). Besides, it has been reported that treatment

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with 50 mg CYP/kg bw continuously for 2 or 4 weeks caused disruption in the estrous cycles in rats (Sangha and Kaur, 2011).

Despite these studies, there is no information regarding the effect of cypermethrin on the ovarian follicular development, effect if any on reproductive parameters of first generation offspring of treated female and reversibility of effects. Moreover, the doses of CYP used were quite higher in earlier studies, whereas very low doses of other pesticides are known to affect reproduction. For instance decrease in the pool of healthy, large and medium follicles with increase in the atretic follicles have been reported following treatment with some pesticides, i.e. chlordecone (Swartz and Mall, 1989) and methoxychlor (Martinez and Swartz, 1991) in mouse and carbofuran (Baligar and kaliwal, 2004a), endosulfan and malathion (Koç et al., 2009) and tetradifon (Badraoui et al., 2010) in rats, thereby indicating a potential reduction on fertility (Swartz and Mall, 1989; Martinez and Swartz, 1991).

Hence, the present study aims at finding out whether or not doses less than 1/100th of LD_{50} of CYP affect ovarian gametogenic and steroidogenic activity, estrous periodicity, and fertility and if so whether the effects are reversible.

2. Materials and methods

2.1. Animals

Seventy adult Swiss albino female mice weighing 33–37 g obtained from inbred population of the central animal facility, University of Mysore were housed in polypropylene cages (5 animals per cage) with husk as the bedding material under 12:12 h light-dark schedule (lights on 7am to 7 pm) at 27 ± 2 °C and 70% humidity. The animals were supplied with standard mice chow pellets and water *ad libitum* during the period of the experimentation. The protocols were approved by Institutional animal ethics committee and the guide lines of CPCSEA, Govt. of India were followed for care and maintenance of animals.

2.2. Chemical and treatment

The doses of CYP used in the present study were based on mouse oral LD_{50} value of technical grade cypermethrin (657 mg/kg body weight; Rose, 1982; WHO, 1989) when used as aqueous suspension. An emulsifiable concentrate of CYP (10%) (Superkiller- 10% EC, Dhanuka Agritech Limited, India) was purchased and diluted in distilled water to get required concentrations i.e. 1.38, 2.76 and 5.52 mg/kg body weight (bw) corresponding to 1/476th, 1/238th and 1/119th of LD_{50} value of the aqueous suspension of CYP respectively. Further, doses were adjusted to the body weight (bw) (average bw 35 g) of the mice used in the experiment. The adult female mice were randomly divided into four groups, controls (20) and three treatment groups (30 in each group). Body weight (initial bw) of each mouse was recorded before commencement of treatment. Control group received 0.1 ml distilled water per mouse, whereas each mouse in 2nd, 3rd and 4th groups received 1.38 (low dose), 2.76 (medium dose) and 5.52 (high dose) mg CYP /kg bw in 0.1 ml distilled water respectively. The mice were administered vehicle or CYP orally (intubation) through a smooth plastic tube attached to a syringe on alternate day for two durations, 6 weeks (D1) and 12 weeks (D2). Body weight of each mouse was recorded at weekly intervals throughout the experimental period i.e. 3 months and expressed as% change in body weight compared to initial body weight. After each treatment period (6 weeks or 12 weeks) five mice in each group were autopsied 24 h after last administration and the weights of the body, ovary, Fallopian tube and the uterus were recorded and later converted into organ weight per 100 g body weight (relative weight). The ovary, fallopian tube and the uterus were removed, washed in normal saline to free from blood and connective tissue and fixed in Bouin's fluid for histopathological study. Blood samples were collected and serum was separated and stored at −80 °C until used to determine serum 17β-estradiol concentration. Five mice of each treated group were used for fertility test and another five mice were maintained for 6 weeks without any treatment and then autopsied to study recovery of CYP effects if any.

2.3. Histology of the ovary and follicular count

The left ovaries were fixed in Bouin's solution, embedded in paraffin wax, Serial sections of 5 μm thicknesses were cut and stained with haematoxylin and eosin. The stained sections were photographed using Olympus digital camera under appropriate magnification to show major histological alterations. The ovarian follicles were classified according to Pederson and Peters (1968) and Cooper et al. (1993). Atretic follicles were identified following morphological criteria described by Greenwald and Roy (1994). A follicle was considered to be undergoing atresia whenever a minimum of 5% pyknotic granulosa cells were found in a single section or the oocyte showed signs of degeneration or thinning of cumulus oophorus (Greenwald and Roy, 1994). The number of healthy and atretic follicles in each category was counted in a serial section of the entire left ovary. Each section of the ovary was observed and only the follicles showing full size oocyte was included in counts of respective category and care was taken not to repeat the counting of the same follicle more than once. Corpora lutea were counted and identified based on presence of hypertrophied luteal cells with rich blood supply giving appearance of a gland.

2.4. Estrous periodicity

During the experimental period vaginal smear of each mouse in all the groups was obtained and observed under light microscope every day (10 AM) and stage of the estrous cycle was determined as per the description of Cooper et al. (1993) based on data of each mouse mean number of estrous cycles per month of each experimental group was computed.

2.5. Fertility parameters

Fertility was determined after each treatment period. Each proestrus female in all the experimental groups (five per group) was left in a cage with normal male mouse with proven fertility. The presence of vaginal plug or spermatozoa in the vaginal smear next day conformed the mating. Percentage of fertility was calculated based on number of females mated and number conceived in each group. The pregnant females were allowed to deliver the pups to find out differences if any in the litter size, litter weight, or gestation period between control and treated animals. Number of females conceived, average length of gestation, litter size, and litter weight of each group were recorded and expressed as mean values of each group. The pups of each group were separated from the mother on post natal day 22. Male and female pups of each group were separated and maintained separately to study the onset of puberty. Starting from PND 23 the pups were examined daily to record the age (in days) at preputial separation in males (i.e. separation of the prepuce from the glans penis) and vaginal opening in females. On the day of vaginal opening a light yellow colored watery liquid emanating characteristic smell appeared in the vaginal region and it was possible to flush saline through vagina to obtain exfoliated cells through a pipette for microscopic observation. Estrous cycles were recorded daily for 2 months following the day of vaginal opening as per the description of Cooper et al. (1993).

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