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Research Article

Gonadotoxicity due to cypermethrin in Wistar albino rats and its effect on the growth and development of their progeny

Priyanka Shuklan
Department of Zoology, Maharshi Dayanand University, Rohtak-124001 (Haryana), India
Sudesh Rani*
Department of Zoology, Maharshi Dayanand University, Rohtak -124001 (Haryana), India

*Corresponding author email id: sudeshrani.zoo@mdurohtak.ac.in

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Abstract
Cypermethrin (CYP) is a synthetic pyrethroid. It acts as an endocrine disruptor which negatively affects animal's reproductive and developmental processes of progeny. So, The present study aimed to examine the toxicity of oral administration of CYP at three different doses (25mg, 50mg, and 75mg/kg) on the reproductive organs of albino rats and also on the growth and development of pups. For this study, animals were divided into four groups: Group I was the control group, and the remaining three were experimental groups that received CYP at a dose of 25mg (group II), 50mg (group III), and 75mg (group IV) per kg body weight (BW) of rat per day for 30 days. There are sixteen animals in each group- eight males and eight females. Histological studies of reproductive organs suggested that CYP exposure caused alteration in the structure of the ovary and testis tissue. In females, significant changes in the ovary, such as distorted follicles, loss of follicular stages, presence of antral follicles, damaged granulosa and theca cells, and blood vessel congestion, were observed, while in males, there were alterations in testes tissue damage in seminiferous tubules, reduced number of germinal cells, and reduction in the size of seminiferous tubules. A significant reduction was observed in the number, length, eyes opening, fur arrival time, and size of pups. Fertility was also recorded up to 80% in the 50 and 75mg CYP-treated female rats. The study suggested that CYP exposure caused gonadal toxicity in albino rats and affected the development of neonates.

Keywords: Cypermethrin, Gonadal toxicity, Histomorphology, Neonates, Reproductive potential

INTRODUCTION

Because of growing knowledge of pesticides' effectiveness in agriculture, public health, post-harvest technology, and animal husbandry, pesticide consumption has expanded globally (Madu *et al.*, 2015). Several pesticides are utilized worldwide, including carbamates, organochlorides, and organophosphorus. Due to their excellent efficacy at low doses, ease of breakdown, and low toxicity to mammals, synthetic pyrethroids are employed most frequently. Pyrethroids are a synthetic form of pyrethrins. Cypermethrin (CYP) is a type II synthetic pyrethroid commonly used as an insecticide in households and agriculture (Singh *et al.*, 2020). CYP mainly acts as a neurotoxin. It causes the opening of sodium channels for a longer duration, resulting in membrane depolarization. As a result, neurotransmitters are released at higher concentrations, which causes the central nervous system to become hyperexcited. Furthermore, CYP exposure influences the activation of voltage-gated calcium, potassium, and chloride channels, all contributing to DNA damage and oxidative stress (Kumar Singh *et al.*, 2012). It can pass the placenta barrier, impacting fetal neurological development and weight (Ramon-Yusuf *et al.*, 2017). In previous studies, synthetic pyrethroids (CYP) are known for their extreme toxicity as they act as an endocrine disruptor (ED). They also have anti-androgenic and anti-estrogenic effects that cause unintended consequences on the non-target organism(Singh *et al.*, 2020). Cypermethrin toxicity causes alteration in the development of the reproductive system and also affects ovulation and implantation in female rats (Rescia and Mantovani, 2007). CYP toxicity also reduced fertility in men (Joshi *et al.*, 2011). Chronic pesticide exposure at low doses in adults can block sex hormones, resulting in aberrant sexual development and teratogenic effects in embryos (Garcês *et al.*, 2020). Furthermore, it was noted that rats' estrous cycles were continuously disturbed after receiving CYP at a dose of 50 mg/kg for two or

four weeks (Sangha and Kaur, 2011). According to earlier research, CYP exposure had a negative impact on the gonads of male and female mammals (mice, rats, and rabbits), and also on their fetuses(Obinna and Agu, 2016). Damage caused in the Male reproductive system is associated with toxicity caused by synthetic pyrethroids (Li *et al.*, 2013). The impairments caused in the reproductive system by synthetic pyrethroids may be related to the anti□androgenic activities of pesticides. Although many authors (Al-Hamdani *et al.*, 2017; Elbetieha *et al.*, 2001) have examined the specific maternal and paternal effects of cypermethrin on offspring, there is a dearth of information about the impact of cypermethrin on the development of offspring when both parents receive cypermethrin treatment. The present study was designed to evaluate the harmful effects of CYP on the histology of albino male and female rats' reproductive organs and to investigate the reproductive capacity of treated rats.

MATERIALS AND METHODS

Chemicals

An emulsifiable concentrate of Cypermethrin (25% EC) (chemical formula $C_{22}H_{19}Cl_2NO_3$, molecular weight: 416.3g/mol) manufactured by Dhanuka Agritech Limited, was procured from Rohtak pesticide store. Distilled water was used in dilutions to get the needed concentration. The doses for the present investigation were selected based on the previous study (Sangha *et al.*, 2013) with a few minor modifications. Therefore, the low, medium, and high doses were determined to be 25 mg, 50 mg, and 75 mg, respectively.

Animals

Sixty-four virgin male and female Wistar albino rats (100-150g), ranging in age from three to four months, were bought from the disease-free small animal house at the Lala Lajpat Rai University of Veterinary and Animal Science in Hisar. They were allowed to stay in plastic polypropylene cages (4 rats/cage) with a proper light -dark 12-12-hour cycle schedule at 25^0C temperature under air conditioning and adequate ventilation during the experiment. Animals were fed with standard laboratory food pellets and *ad libitum* to drinking water. They were acclimatized for one week under standard laboratory conditions. The animals were divided into distinct groups following the acclimatization phase.

Ethical approval

The Institutional Animal Ethical Committee (IAEC) of Maharshi Dayanand University (MDU) Rohtak has approved the protocols used in this study. Care and maintenance of animals were done according to the guidelines of CPCSEA, Govt. of India.

Experimental design

Four groups were made to carry out the present study. In each group, 16 animals (8 males and 8 females) were present.

Group I was taken as a control group, receiving no cypermethrin exposure. The experimental groups were designated as Groups II, III, and IV. The experimental group rats received CYP orally at doses of 25mg, 50mg, and 75 mg/kg according to their body weight (BW for one month (Fig. 1).

Doses were freshly prepared daily, dissolved in water, and then given according to the body weight of the rats. Animals were observed closely throughout the experiment. After dosing for one month, 6 animals (3M and 3F) from each group were used for the histological study of the reproductive organs (testes and ovaries), and the remaining animals (5M and 5F) from each group were used for the analysis of the reproductive potential of animals.

Histomorphological analysis

Animals were sacrificed after the experiment, and the ovary and testes were taken out and put in a 10% formalin solution. For slide preparation, organs were cut into small pieces and washed under running tap water. After that tissue was dehydrated in an ascending series of alcohol. The tissue was cleared in xylene. The blocks were prepared and 5µm tissue sections were prepared by using a semi-automatic microtome, and then hematoxylin and eosin (H&E) were used to stain

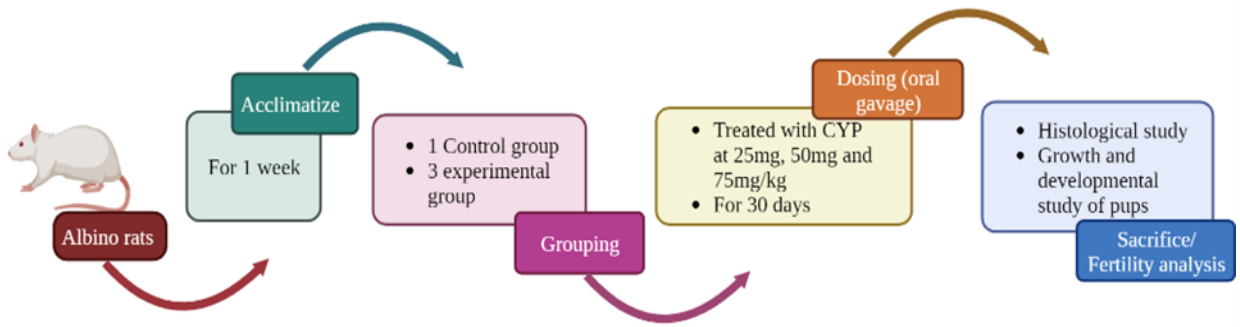

Fig. 1 *Experimental design of present study*

the slides and then viewed under a light microscope for histomorphological study (Chauhan & Rani, 2024).

Reproductive potential study

After a treatment of 30 days, control and cypermethrin-treated male and female albino rats were allowed to mate to check whether CYP-treated male and female rats were reproductively active. For that 5-mating pair was made in each group and the male and female were kept in one cage in a ratio of 1:1. The vagina of the female was checked daily for the vaginal plug. The presence of spermatozoa in the females' vaginal fluid confirmed that they had successfully mated. After mating was confirmed, the male rat was separated from the female, and the pregnant female was allowed to deliver the pups in the well-spaced cage. The reproductive potential was calculated based on the number of mated and fertilized females in each group. The growth and development of pups were checked in terms of litter weight, litter size, the opening of eyes, and the arrival of fur in control and treated groups. The number of females conceived, litter weight, and litter size were recorded for each group(Al-Hamdani *et al.*, 2017).

Statistical analysis

The data was shown as mean ± SE. Tukey's post hoc comparisons test and one-way analysis of variance (ANOVA) were utilized to assess the statistical significance of variations in the mean values of the experimental and control groups. The difference was statistically significant at $p \leq 0.05$ and $p \leq 0.01$.

RESULTS

Histomorphological analysis of gonads

Effect on ovary

Control (Group I) female rats represented the standard structure of all stages of follicles, oocytes (Fig. 2A), interstitial cells, granulosa, and theca cells. Low-dose-treated (Group II) females (25mg) showed the presence of Atretic follicles, distorted follicles, and congestion of blood vessels (Fig. 2B). 50mg treated (group III) females showed the presence of Atretic follicles, distorted primary follicles (Fig. 2C), and congestion of blood vessels. High-dose (75mg) treated (group IV) female rats showed more atretic follicles, distorted follicles, loss of follicles, increased antral follicles, damaged granulosa cells (GC) and theca cells (TC), and congestion of blood vessels (Fig. 2D).

Effect on testes

In the male testes of the control (group I), the normal structure of seminiferous tubules and spermatids (Fig. 3A) was noticed. In 25mg CYP treated (group II), males reduced size of seminiferous tubules (Fig. 3B) at some sites was observed. 50mg CYP-treated (group III) male showed deformed seminiferous tubules, a reduction in the number of germinal cells (Fig. 3C), Leydig cells, and Sertoli cells. In comparison, males treated with 75mg CYP (group IV) showed a smaller size of seminiferous tubules, loss of seminiferous tubules, a decrease of interstitial cells, and widening of the lumen of seminiferous tubules (Fig. 3D).

Reproductive potential analysis

Fertility test

All females in the control as well as an experimental group, were found to be reproductively active. However, 100% fertility was observed in the control and 25mg CYP treated female groups. On the other hand, fertility was observed up to 80% in medium (50mg CYP treated) and higher doses (75mg CYP treated) female groups (Table 1).

Gestation period

The experimental group of females showed a significant increase in their gestation period compared to the control group of females. Further, higher dose (75mg)

Table 1 Reproductive potential study in control and CYP-treated rats

Parameters	Control (Group I)	Low dose (25mg) (Group II)	Medium dose (50mg) (Group III)	High dose (75mg) (Group IV)
No. of females allowed to mate	5	5	5	5
No. of females conceived	5	5	4	4
% of fertility	100%	100%	80%	80%
Gestation period (days)	21.4 ± 0.24	22.0 ± 0.32**	23.2 ± 0.37**	24.0 ± 0.32**
Number of pups	9.0 ± 0.37	8.0 ± 0.32**	7.0 ± 0.37**	6.0 ± 0.32**
Mortality % in pups	0%	8.33%	15%	27%
Fur arrival in pups (days)	8.6 ± 0.24	9.4 ± 0.24*	9.8 ± 0.37*	10.6 ± 0.68*
eyes opening in pups (days)	17.0	17.8 ± 0.2**	18.4 ± 0.51**	19.0 ± 0.45**

Values are represented as mean± SE. Mean values of the control and experimental groups are compared through one-way ANOVA and Tukey's test. * Values are significantly different at $p < 0.05$; ** Values highly differ significantly ($p < 0.01$) from the control group

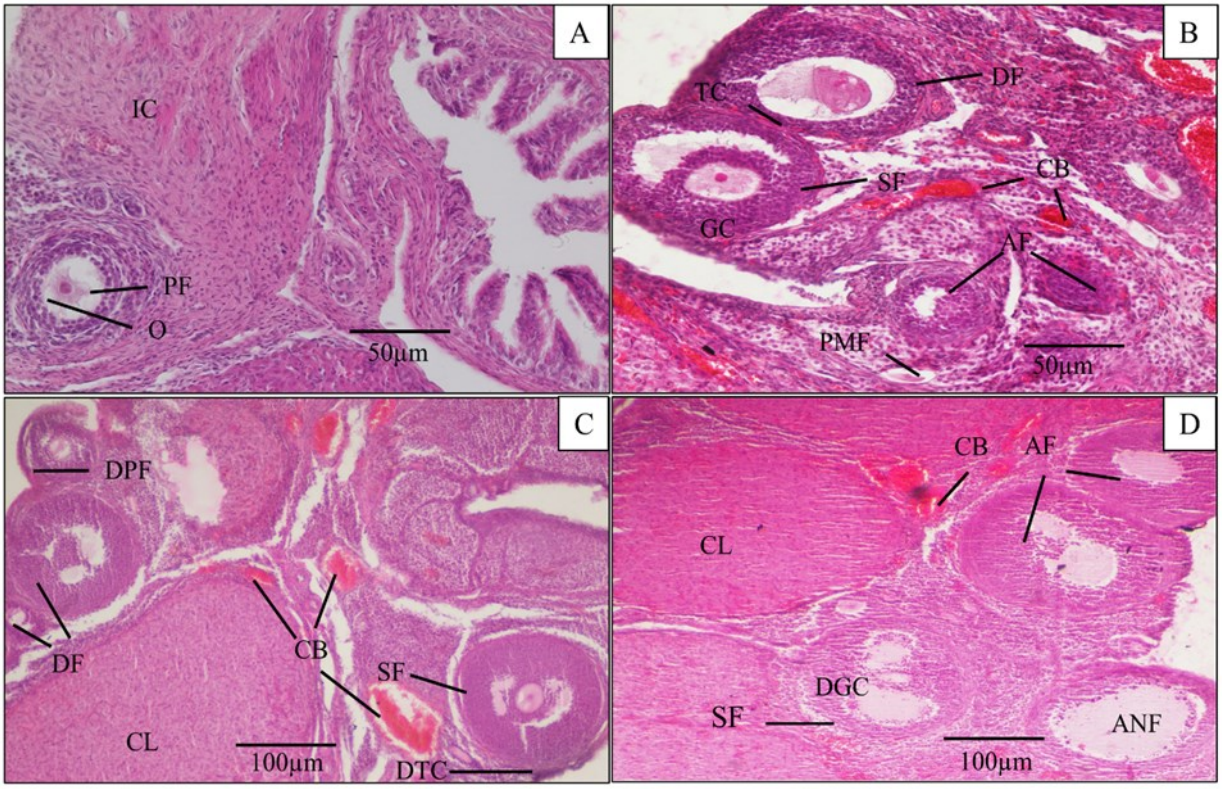

Fig. 2. *Ovary section of female rats stained with H&E, (A) control group: showing normal structure of all stages of follicles, 200X (B) 25mg CYP group showed distorted follicles and congestion of blood vessels, 200X (C) 50mg CYP group: Atretic follicles and damaged follicles, 100X (D) 75mg CYP group: loss of follicles, and damaged GC and TC were seen in treated groups, 100X. PF- primary follicles, O- oocyte, IC- interstitial cells, DF- damaged follicles, SF- secondary follicles, GC- granulosa cells, TC- theca cells, AF- Atretic follicles, PMF- primordial follicles, DPF- damaged primary follicles, CB- congestion of blood vessels, DTC- damaged theca cells, CL- corpus luteum, DGC- damaged granulosa cells, ANF- antral follicles*

treated females' values for this parameter were found to be more significant ($p < 0.01$) as compared to control and lower dose CYP treated females (Table 1).

Number of pups

The average number of pups delivered by the females of the control group remained high. On the other hand, the average number of pups was significantly ($p < 0.01$) reduced in CYP-treated females. The females treated with 75mg of CYP showed the greatest decrease in the average number of pups (Table 1).

Growth and development of pups

The pups on post-natal day 1 (PND 1) in control, 25mg CYP, 50mg CYP, and 75mg CYP treated females were slightly different in appearance (Fig. 4).The body weight of pups was significantly ($p < 0.05$) decreased in all treated groups (Fig. 5). A considerable ($p < 0.05$) decrease in the length (Fig. 6) and size of pups was also observed in all CYP-treated female groups. The body fur arrival time and eyes opening were slightly delayed in the experimental group compared to the control group. The growth of 75mg CYP-treated female pups was comparatively lower than the lower dose-treated groups. Body texture, size, and behavioral activity were also different in the CYP-exposed group compared to the control group (Fig. 4). Mortality was also reported in the experimental group's pups, which was recorded as high in the 75mg CYP-treated group.

DISCUSSION

Significant changes were noticed in the histomorphology of the gonads of albino rats. Female albino rats, when treated with cypermethrin for 30 days, showed histological alteration in the ovary tissue (Fig. 2), such as Atretic follicles, degenerated follicles, and congestion of blood vessels (Groups II, III and IV). Cypermethrin treatment caused a reduction in healthy follicles and an increase in atretic follicles. This statement was found to be in agreement with the reports given by the authors regarding the effect of different pesticides on different animals (Guerra *et al.,* 2011; Singh *et al.*, 2020). According to Grewal *et al.* (2010), rats exposed to CYP lost follicular cells and oocytes in their ovaries. Due to CYP intoxication, the earliest antral stage of ovarian follicles was considered to be the stage of ovary tissue that was most affected (Sangha *et al.*, 2013).

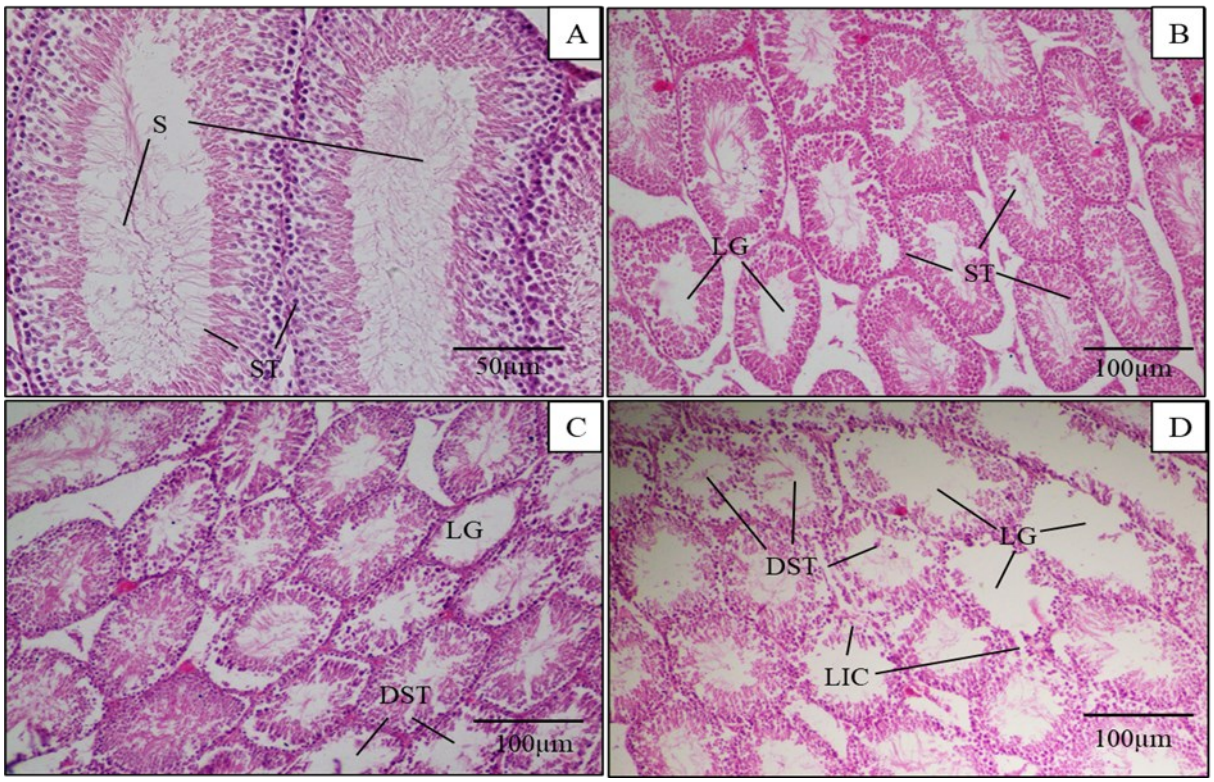

Fig. 3. *Testes section of male rats stained with H&E, (A) control group: showing a normal structure of spermatids and seminiferous tubules 200X (B) 25mg CYP group: absence of spermatozoa was observed, 100X (C) 50mg CYP group: loss of spermatocytes and damaged seminiferous tubule was noted, 100X(D) 75mg CYP group: loss of some stages of spermatogenesis were seen 100X. S- spermatozoa, ST- seminiferous tubule, LG- loss of germinal cells, DST- distorted seminiferous tubule, LIC- loss of interstitial cells*

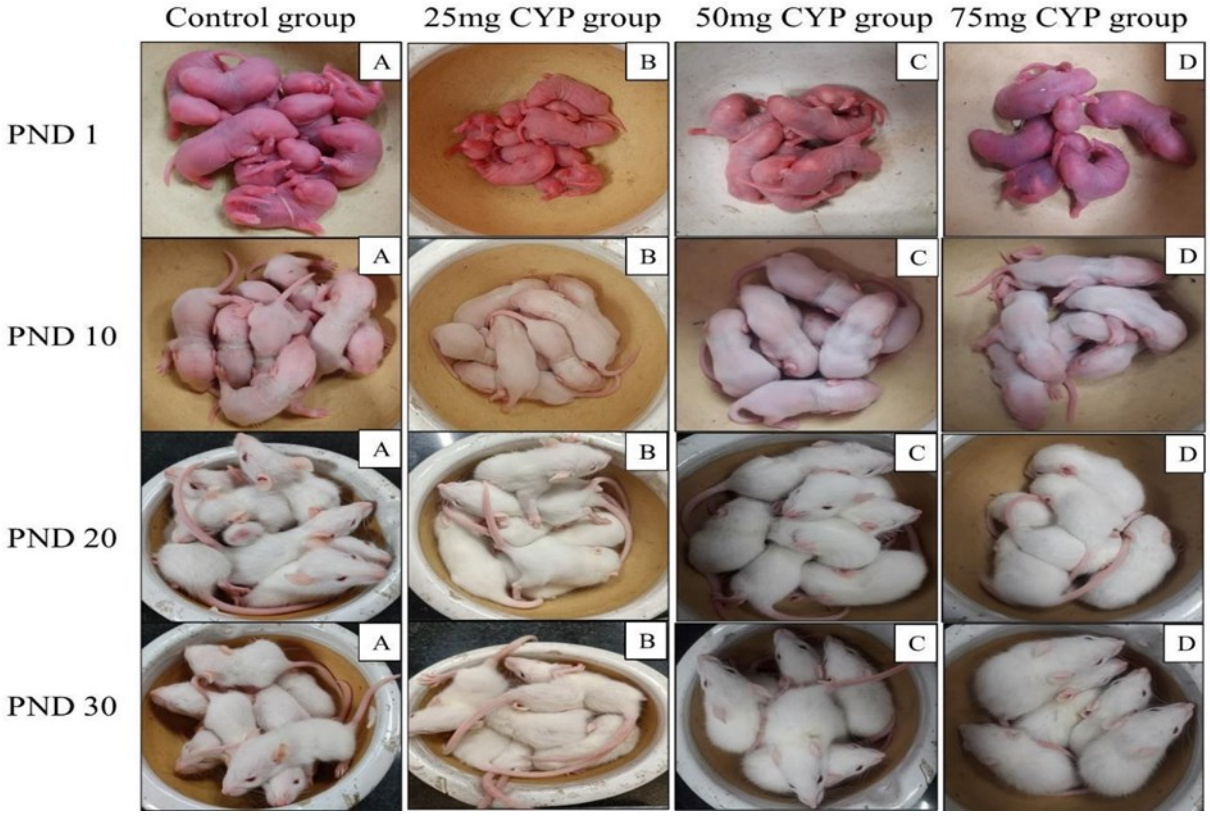

Fig. 4. *Representing the morphological appearance of pups on PND 1, 10, 20 and 30 (A) Control female pups (B) 25mg CYP treated female pups (C) 50mg CYP treated female pups (D) 75mg CYP treated female pups.*

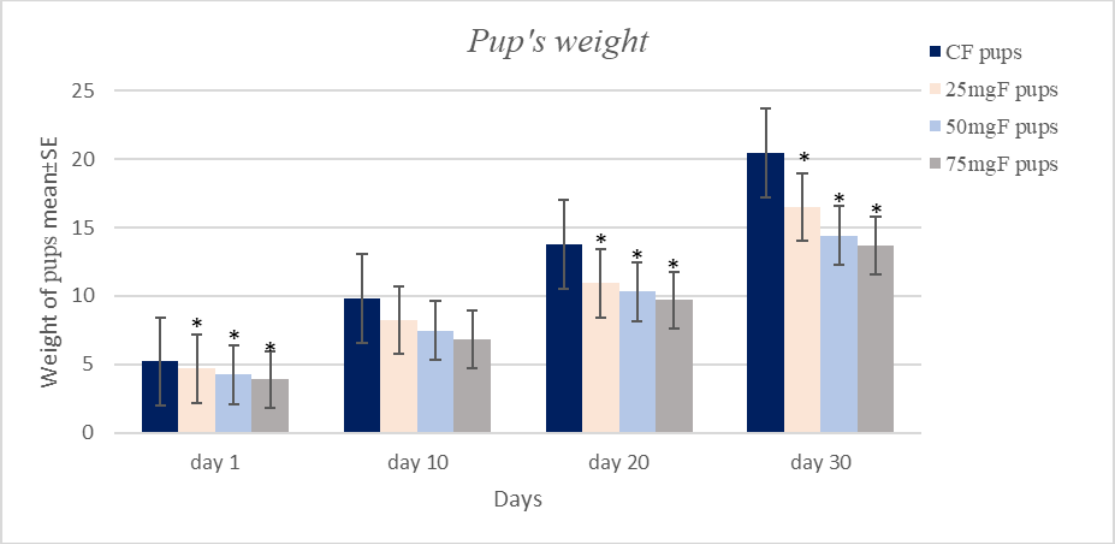

Fig. 5. *Data represented the mean pups' weight ± SE of CYP-treated and control groups. * Mark values significantly different (p > 0.05) from the control group; values (p > 0.05) do not exhibit statistical significance*

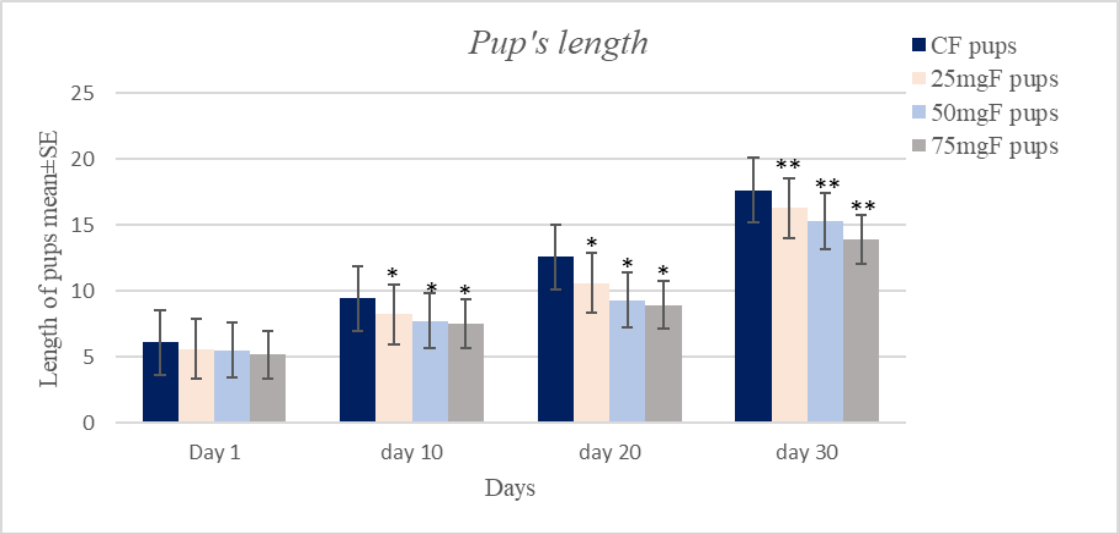

Fig. 6. *Data represented as the mean length of pups ± SE of CYP-treated and control groups. * Values significantly different (p > 0.05) from the control group. **p <0.01 values highly differed significantly from the control group;Values (p > 0.05) do not exhibit statistical significance*

Similarly, CYP treatment increased the atresia of the antral follicles by directly affecting ovarian tissue or indirectly changing endogenous hormones (Bretveld *et al.*, 2006). Cypermethrin treatment at a very low dose caused a reduction in primordial follicles, which indicates that exposure to cypermethrin causes a reduction in the reproductive potential of mammals. Mammals have a set number of primordial follicles at birth, and as these follicles are the source of all subsequent follicle development, their destruction would result in the cessation of ovarian cycle progression((Al-Hamdani *et al.,* 2017). Therefore, exposure to CYP and other pesticides reduces the reproductive potential of mammals. Histological analysis of male albino rats after repeated exposure to cypermethrin at different doses (25mg, 50mg, 75mg/kg) causes dose-dependent alteration in the structure of testes (Fig. 3). The study of Joshi *et al.* (2011) found that the rats exposed to cypermethrin with different doses such as 50 or 75mg for 45 days showed a reduction in the size of the seminiferous tubule, increased intertubular space, ruptured interstitial cells, and complete arrest of spermatogenesis, was found to be comparable to the current observation regarding the adverse effect of cypermethrin on testes. Grewal *et al.* (2010) also suggested that CYP intoxication damaged the seminiferous tubules and altered the histoarchitecture of testes. According to Elbetieha *et al.* (2001), oral ingestion of CYP displayed a decline in the number of seminiferous tubules, accumulation of connective tissue between seminiferous tubules and the release of premature spermatozoa in the lumen of seminiferous tubules was also observed in albino rats. Deltamethrin exposure in rats caused alteration in the structure of testes, such as distorted seminiferous tubules, thicken-

ing of the basement membrane, and irregular structure of seminiferous tubules (Kumar and Nagar, 2014). Chouabia *et al.*, 2021 also reported alteration in the stages of spermatogenesis after treatment with CYP for 28 days. Similar results were observed in the studies of (Ahmad *et al.*, 2012 and Li *et al.*, 2013).

The present findings indicate that the growth and development of pups as well as the fertility of male and female albino rats both affected by CYP exposure. The current observation was also supported by Al-Hamdani *et al.*, (2017) who suggested that cypermethrin exposure for two durations (6 and 12 weeks) with different doses reduces the reproductive potential of mice and similarly also reduces the body weight, length, and size of pups. Another study also reported a decrease in pups body weight and size (Obinna & Agu 2016). Females treated with cypermethrin and dimethoate during gestation showed increased foetal resorption and lower litter weight and size (Madu, 2015; Ramon-Yusuf *et al.*, 2017). Elbetieha *et al.* (2001) also reported that when females mated with CYP-exposed rats, there was a significant reduction in the number of implantation sites, pregnancy rate, and viable foetuses observed. CYP intoxication for a longer duration (12 weeks) also adversely affects fertility and reproduction in males. The current study disclosed that CYP exposure caused a significant reduction in the eye-opening and fur arrival period of treated female pups.

Conclusion

The present study concluded that CYP exposure affected adult albino rats and their progeny. CYP exposure altered the histoarchitecture of the ovary and testes of albino rats and adversely affected the animal reproductive potential. Along with this CYP intoxication negatively affected the growth and developmental parameters of the pups. Hence, the toxicity of pesticides is hazardous to the fertility of domestic animals and humans. Therefore, it is advised that people (principally pregnant females) in direct/indirect contact with such pesticides must be aware of the adverse effects/ consequences on their health. They also need to be guided about the proper handling of all the things related to the working criteria in the context of these risky pesticides.

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Conflict of interest

The authors declare that they have no conflict of interest.

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