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IMIDACLOPRID INDUCED REPRODUCTIVE TOXICITY IN FEMALE ALBINO RATS

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ABSTRACT

Imidacloprid a systemic neonicotinoid, mainly used for controlling insects and pests in agricultural sector. Two doses of imidacloprid (10 and 20 mg/ kg/ day) were selected based on LD_{50} and given through oral intubation to female rats for 60 days. Disturbed cyclicity with significant ($P<0.05$) decrease in the number of estrous cycle days and increased diestrous index was observed at high dose. Serial sections of ovary were studied for atretic follicles in imidacloprid treated rats. Number of healthy follicles was significantly ($P<0.05$) decreased with increased number of atretic follicles at high dose of imidacloprid. The histopathology of ovary also revealed more atretic follicles at high dose of imidacloprid. Decreased body weight and ovary weight in imidacloprid treated rats was dose dependent. Based on the observed effects, it can be concluded that imidacloprid produced more significant effects on female reproduction at high dose (20 mg/ kgbw/ day) as compared to low dose (10 mg/ kgbw/ day).

Key words: Imidacloprid, albino rats, ovary, growth, follicles, high dose, low dose, body weight, ovary weight, atretic follicles, healthy follicles, reproduction, cyclicity

Pesticides are used in agricultural sector to control insect pests and these raises a number of environmental distresses (Bretveld et al., 2006). Pesticide remnants can move into food web and after a permissible limit the hazards due to pesticide usage is of great concern (McLachlan, 2001). Pesticide poisoning is a major threat to the developing countries, leading to death of many people each year (Dawson et al., 2010). The pesticide usage has increased with the increased awareness of their utility among people (Muthviveganandavel et al., 2008). Imidacloprid (IM), a chloro-nicotinyl is a broadly used neonicotinoid for crop safety throughout the world (Chao and Casida, 1997). Because of its high pesticidal activity at a very small amount (Broznil et al., 2008) it is commonly used to control the pests of various crops throughout the world (Proenca et al., 2005). Metabolism of imidacloprid is done by human cytochrome P_{450} isozymes by hydroxylation and reduction reactions (Schulz et al., 2002). Various toxicity studies in animals and humans evidenced or suggested that these insecticides may cause various brain disorders, tumors growth, reproductive and respiratory disorders (Holmstrup et al., 2010; Yang et al., 2008, Whitehorn et al., 2012). Based on LD_{50} of imidacloprid that is 450 mgkg/ day it is classified as moderate toxic insecticide by Environment Protection Agency (EPA) (Bhardwaj et al., 2010; Brunet et al., 2010)

A number of studies indicated the toxic effect of imidacloprid as immonotoxic, genotoxic, neurotoxic and developmental effects of imidacloprid (Lonare et al., 2014; Gawade et al., 2013). Some studies reported that pesticide exposure can affect the reproduction by interferring with neural transmission and reproductive endocrinology which may alter the functioning of reproductive organs (Gill et al., 2011). From the past few studies a number of evidences have been reported which showed the toxic effects of pesticides or environmental contaminants on male and female reproductive system (Aitken et al., 2016). Similar studies on pesticides and insecticides proved the effect of these pesticides on female reproduction and folliculogenesis (Ding et al., 2020; Gonsioroski et al., 2020). Despite a lot of information on imidacloprid toxicity studies, very scarce or no information is available on the effect of imidacloprid on follicular development in the ovary. The present study mainly aims at finding out the toxic effect of 10 mg/ kgbw/ day (low dose) and 20 mg/ kgbw/ day of imidacloprid (high dose) treatment on estrous cyclicity, ovarian weight and damage in ovarian follicles of female albino rats.

MATERIALS AND METHODS

The experiment was done in Animal Physiology Laboratory of Department of Zoology, Punjab Agricultural University, Ludhiana. Technical grade of imidacloprid was purchased from Ludhiana, India. For this study, female albino rats, aged 3 months, weighing between 100–150 g procured from Guru

Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. The rats were kept in cages and were given free access to pelleted food and water ad libitum. The rats were given temperature (22± 2°C) and humidity (30–70%) and lightning schedule with 12 hr light and dark cycle. The protocol for this experiment follows the guidelines for proper caring and use of animals in the laboratory research and got approved by Institutional Animal Ethics Committee. Test concentrations were achieved by dilutions of imidacloprid with corn oil. Active ingredients (% age) of formulation of imidacloprid was used to calculate the test concentrations. Dose of imidacloprid was selected based on its LD_{50} which is 450 mg/ kg body weight (Bomann, 1989). Group I -control, Group II (T1) -10 mg/ kg/ day of imidacloprid (low dose), Group III (T2) - 20 mg/ kg/ day of imidacloprid (high dose). The estrous cyclicity of control and treated rats was checked by examining the vaginal smears every morning. Rats were sacrificed after the treatment of 60 days.

For histopathology, ovaries were excised from control and treated rats and fixed in 10% formalin. Then each tissue was processed and dehydrated, paraffin sections of 5 µm were cut and staining of tissues was done with haematoxylin-eosin stain and examined under microscope. Serial sections of control and treated ovary were studied for various interpretations like total number of healthy follicles, number of atretic follicles, corpus luteum (Kaur and Guraya, 2003). From the serial section of ovary, the follicle number in every developmental stage was counted. The follicles were divided into six groups according to Cooper et al., (1993) and Pederson and Peters (1968) i.e. primordial, primary, secondary, tertiary, early antral, antral follicles and corpus luteum. Primordial follicle consists of an ovum or oocyte surrounded by granulosa layer of cells having squamous shape. Primary follicle consists of large oocyte surrounded by two layers of granulosa cells having cuboidal shape. Secondary follicle consists of oocyte surrounded by more than two or two to three granulosa cell layers having cuboidal shape. Early antral follicle consists of oocyte surrounded by three or more granulosa cell layers with no cavity (antrum). Antral follicle consists of an oocyte surrounded by many granulosa cells layers having antrum. Ovary weight, estrous cyclicity, number of follicles were analysed using Graph Pad Prism. Statistical analysis was done by analysis of variance (ANOVA) between control and treated group of rats followed by Dunnet's test.

RESULTS AND DISCUSSION

High dose (20 mg/ kgbw/ day) of imidacloprid treated rats showed decreased ovarian weight and body weight. This decrease in body weight and ovary weight was not significant at 10 mg/ kgbw/ day as shown in Table 1. Decreased body weight may be due to decreased feed intake. Decrease in weight of ovary may be due to toxic effect of imidacloprid on ovary (Yavasoglu et al., 2006). Similar results have also been shown after treatment with organophosphates (methyl parathion) that there was decrease in the ovarian weights (Kaur and Dhanju, 2005). Vohra and Khera (2016) also showed similar decrease in ovary weight after exposure of imidacloprid to female rats in three generation study. The present study showed that control rats have normal 4-5 days estrous cycle while high dose (20mg / kgbw/ day) treated females showed significant decrease in estrous cycle days with significant increase in diestrous phase. There was increase in diestrous index in both the doses of imidacloprid treated rats as shown in Table 1. This disturbed cyclicity was due to disrupted reproductive endocrinology (Bretveld et al., 2006). Borgeest et al., (2002) also reported significant increase in estrous phase and decrease in number of estrous cycle days after treatment with methoxychlor in mice. Vohra and Khera (2018) also reported disruption in estrouscyclicity after exposure of high and low dose of imidacloprid to female wistar rats in two generational study. Similar results have also been shown by Baligar and Kaliwal (2002) when rats were treated with

Table 1. Imidacloprid induced changes in estrous cyclicity, relative ovary weight and net body gain weight of female rats

	Control	T1	T2
No. of cycles	5.6±0.22	4.2±0.20*	4.31±0.31*
Diestrous index	40.67	51.23	63.45
Ovary weight	0.03 ±0.002	0.029 ±0.001	0.02±0.001*
Body weight gain(g/ 100g bw)	45 ± 4.47	35 ± 6.32	42.5 ± 5.12

Values represent mean± SE of 6 animals in each group. *Significant difference (p= 0.05)
Diestrus index =Days with clear diestrus smear/ 60 days (duration of treatment) x 100.

carbofuran showed decrease in number of estrous cycle days and increase in diestrous phase. Another study has also showed similar results after treatment with organophosphate pesticides (fenthion, dimethoate and methyl parathion) to female rats (Budreau and Singh, 1973; Soratur and Kaliwal, 2000; Maths, 1998). Since imidacloprid is a neonicotinoid insecticide, it may act on hypothalamus in the brain which has effect on ovary and can affect the estrous cyclicity and follicle formation. It can also be due to imbalancing of reproductive hormones after the imidacloprid treatment (Radhika and Kaliwal, 2002).

The kinetics of follicles in the ovaries of treated and control rats were studied under light microscope in present study as shown in Fig. 1 and 2. Control group showed higher number of healthy follicles while high dose of imidacloprid treated rats showed decreased number of healthy follicles in the ovaries. Basic functional unit of mammalian ovary is the follicles. Assessment of number of follicles is an indicator of normal and damaged follicles in the ovary. Two important hormones involved in follicular development are the Follicle Stimulating Hormone (FSH) and Luteinising hormone (LH) (Plowchalk et al., 1993). The present study showed the increased number of atretic follicles and decreased number of normal follicles in higher dose (20 mg/ kgbw/ day) of imidacloprid treated group but no significant effect on healthy and atretic follicles was shown in lower dose of imidacloprid treated female rats (Fig. 3). Similar study conducted by scientists after treatment with cypermethrin showed reduction in number of estrous cycle days, increased atretic follicles and decreased healthy follicles in cypermethrin treated groups as compared to control (Nada et al., 2017). Similar results have been reported by some studies on treatment with different carbamates and chlorinated pesticides. These pesticides reduce the healthy follicles as compared to atretic follicles (Martinez and Swartz, 1991; Jadaramkunti and Kaliwal, 2019). One study have reported to inhibit the development of antral follicles and antrum and in turn resulted in increase in number of atretic follicles (Ataya et al., 2008). It has also been reported by Baligar and Kaliwal (2002) that rats treated with mancozeb also showed similar trend of healthy and atretic follicles.

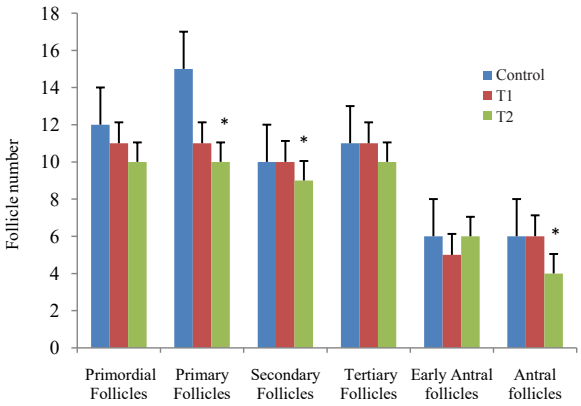

Fig. 1. Effect of imidacloprid on healthy follicles at different stages, at 10 and 20 mg/ kdbw/ day dose. Fig shows the more healthy follicles in control as compared to treated group at $p<0.05$

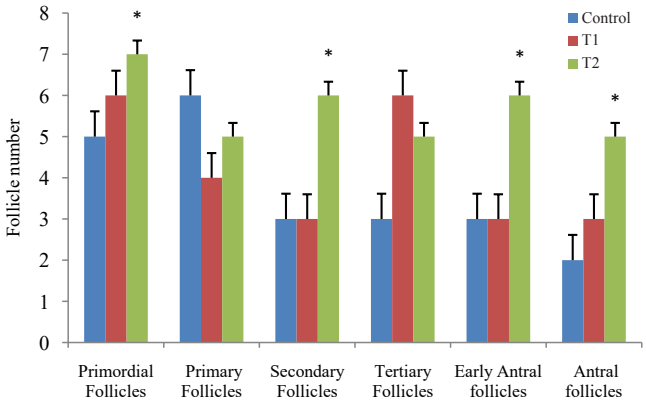

Fig. 2. Effect of imidacloprid on atretic follicles at different stages, at 10 and 20 mg/ kdbw/ day dose. Fig shows the more atretic follicles in high dose of imidacloprid treated rats as compared to treated group at $p<0.05$

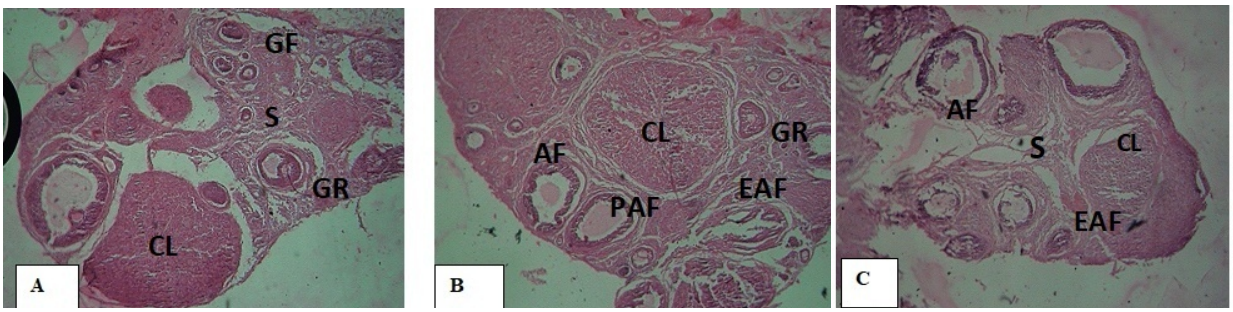

Fig. 3. Ovary section- Control rats (A), T1 (10 mg/ kgbw/ day) (B) and T2 (20 mg/ kgbw/ day) (C) of imidacloprid treated rats: S-Stroma, A-Antral follicles, CL- Corpus luteum, GR- Graffian follicle, GF-Growing follicle, EA-Early antrum, AF-Antral follicle Type, PAF- Pre antral follicle

Similar results have also been observed in mice treating with herbicide atrazine (Pernot et al., 2017)

The histopathological slides of ovaries of control rats showed number of developing follicles i.e. primordial, primary, secondary, tertiary, early antral, antral and atretic follicles. Ovaries from rats treated with 20 mg/ kgbw/ day showed more number of all phases of atretic follicles i.e. antral follicles, corpus luteum, early antrum, antral follicle type and preantral follicle as compared to ovaries of rats treated with 10 mg/ kgbw/ day. Atresia is the breakdown of follicles in the ovary and oxidative stress may also be responsible for increased atretic follicles (Fig. 3). This is authenticated by the findings of Gupta et al., (2006). Similar findings have also been reported by Guneyet al., (2007a, b) after exposure of methidathion to female rats. Similar results have also been reported by Borgeestet al., (2002) when mouse were treated with methoxychlor showed follicular atresia and affects ovarian physiology. Disturbed estrous cyclicity and ovarian follicles is due to direct effect of insecticide on hypothalamic-hypophysial ovarian axis causing reproductive hormone imbalancing. In the present study disruption in estrous cyclicity, decreased number of healthy follicles, increased atretic follicles may be due to damage by the insecticide at hypothalamo-pituitary gonadal axis. Because insecticides or pesticides may destroy reproductive endocrinology (Stoker et al., 2003). Imidacloprid treated rats showed dose dependent toxicity in relation to body weight. In high dose of imidacloprid (20 mg/ kgbw/ day) treated rats significantly decreased body weight gain and decreased ovarian weight was observed in our study as compared to control. In conclusion this study revealed the effectiveness of high dose of imidacloprid (20 mg/ kgbw/ day) to affect estrous cyclicity and atretic follicles as compared to low dose of imidacloprid (10 mg/ kgbw/ day) treated rats.

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CONFLICT OF INTEREST

There is no conflict of interest.

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