Applied Biological Research 20(1): 62-67; (2018)
DOI: 10.5958/0974-4517.2018.00007.1

IMIDACLOPRID INDUCED TOXICITY IN OVARY OF FEMALE WISTAR RATS IN TWO GENERATIONS

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(Received 12 May, 2017; accepted 21 January, 2018)

ABSTRACT

In present study the effect of oral administration of imidacloprid over two generations on the ovary of female Wistar rats was assessed. Female rats were divided into 3 groups. Group 1, as control, was given corn oil only while imidacloprid was administered in group 2 and 3 @ 10 and 20 mg kg^{-1} bw day^{-1}, respectively. A disruption in estrous cyclicity was observed with a prolonged diestrous in both F_1 and F_2 generation. The weight of ovary decreased significantly in treated female rats of F_2 generation. Histopathology of ovary of groups 2 and 3 revealed different stages of follicles. Percentage atresia of follicles was found higher in treated rat ovaries as compared to their respective control rats in both the generations. The results indicate that no observed effect level of imidacloprid for two generations female reproductive toxicity is 10 mg kg^{-1} bw day^{-1}.

Key words: Histopathology, imidacloprid, ovary, phosphatases, rat

INTRODUCTION

The pesticide use has increased with the increase in awareness about its benefits in agriculture and animal husbandry, post-harvest technology and in public health (Milne, 2000). However, pesticides may cause reproductive toxicity through several mechanisms; direct damage to structure of cells, interference with biochemical processes necessary for normal cell function and biotransformation resulting in toxic metabolites (Colborn, 1998, Mclachlan, 2001, Agrawal and Sharma 2010). The level of biochemical constituents in various organs represent the intensity of toxic action of pesticides (Rathod and Kshirsagar, 2010). Endocrine disruptor compounds mimic the structure of natural hormones found in animal/human body and their adverse effects on adult reproductive system are related to their ability to interfere with sex steroid action (Kumar *et al*., 2008).

Imidacloprid (IMI) is most widely used neonicotinoids insecticide in agriculture with registration for use on over 140 crops in over 120 countries (Whitehorn *et al*., 2012). Such a large-scale use can exaggerate the toxic properties and adverse effects of insecticide and can be fatal for human as well as animal health. The study of acute toxicity of imidacloprid following occupational, accidental or suicidal ingestion indicated mild clinical effects such as tachycardia, nausea, vomiting to severe respiratory failure, seizures and even death in human (Proenca *et al*., 2005; Agarwal *et al*., 2007). A case of acute poisoning was reported in human following ingestion of a pesticide formulation containing 10% imidacloprid (Wu *et al*., 2001) and two fatal intoxication cases have been reported recently (Proenca *et al*., 2005). Although it is widely used worldwide, there is still less work done related to its toxicity in female rats. Therefore, this study was designed to evaluate the histological and ultra-structural alterations in testicles induced by imidacloprid in male rats.

MATERIALS AND METHODS

Commercial formulation of imidacloprid (Confidor, 17.8% w/w) used in this study was purchased from the local market in Ludhiana, India.

Animal's husbandry

The study was conducted in the years on sexually mature female albino rats, 3 months of age, weighing 100-150 g obtained from (GADVASU), Ludhiana (India). The animals were housed in groups of two rats per cage. The rats were acclimatized for one week before using them for experimentation. The rats were maintained under controlled conditions of temperature (22±2°C) and humidity (30-70%) with 12 h light and dark cycle. The animals were given standard diet containing pelleted food and water *ad libitum*. The experimental protocol met the national guidelines on the proper care and use of animals in the laboratory research. The Institutional Animal Ethics Committee approved this experimental protocol in XIV meeting of Animal Ethical Committee held on 19.7.12 (approval issued vide letter No. VMC/12/3901-35 dated 6.8.2012).

Adequate dilutions of imidacloprid were made with corn oil to achieve the test concentrations. The test concentrations were calculated from the percentage of active ingredients of the commercial formulation. Eighteen female rats were divided into three groups. Group 1 served as control and was given corn oil through oral gavage. Group 2 was treated with $1/45^{th}$ LD_{50} of imidacloprid. Group was 3 treated with $1/22^{th}$ LD_{50} of imidacloprid. LD_{50} of imidacloprid is 450 mg kg^{-1} bw. Dams and their offspring were treated with imidacloprid during the periods of mating, gestation and lactation. The offspring of different dams in a group of each generation were mated among themselves throughout two generations. F_1 and F_2 generations were attained by the same procedure. Vaginal smears of rats were checked daily for cyclicity during the experimentation. For this, vaginal secretion was collected with a plastic dropper containing 0.9 % saline by gently inserting the tip into rat vagina. Vaginal fluid was placed on glass slides and observed under the light microscope with 10x objective lens to analyze the proportion among three cellular types (leucocytes, epithelial and cornified cells) present in the vaginal smear. The proportion among them was used for the determination of the estrous cycle as described by Mandl (1951).

F_1 and F_2 generation female rats were sacrificed. The reproductive organs *viz.*, ovary, oviduct, uterus and vagina were excised, cleaned off the adhering tissue and weighed separately.

Processing of tissues for histopathology

All F_1 and F_2 rats were weighed and sacrificed by chloroform anesthesia. For histopathological studies, ovaries of treated and control rats were fixed in 10% formalin. After routine processing and dehydration of each tissue, paraffin sections of 5 µm were cut and stained with hematoxylin-eosin for microscopic examination. Serial sections of ovary were studied for various observations like total number of follicles, number of normal and atretic follicles, oocyte and nucleus as described by Kaur and Guraya (1983).

Biochemical analysis

All F_1 and F_2 rats were weighed and sacrificed by chloroform anesthesia. After sacrificing, blood samples were collected directly from heart of F_1 and F_2 rats, drawn into heparinized tubes and plasma was separated by centrifuging at 3000 rpm for 10 min at room temperature. For biochemical studies, ovaries were homogenized in 2 mL phosphate buffered saline (PBS, pH 7.4) and ovarian homogenate was centrifuged at 5000 rpm for 10 min. Alkaline phosphatase (AKP) and acid phosphatase (ACP) activity was measured as described by Bessey *et al*. (1946).

Biochemical analyses were presented as the mean ± standard error of means (SEM). Comparisons were made between control and treated groups using ''analysis of variance (ANOVA)'' as a Statgraphics statistical package (Vohra *et al.,* 2014).

RESULTS AND DISCUSSION

Disturbed estrous cyclicity was observed in higher dose (20 mg kg^{-1} bw day^{-1}) of imidacloprid treated rats with significant increase in the duration of diestrous phase in both F_1 and F_2 generations. Disruption in cyclicity was not significant at lower (10 mg kg^{-1} bw day^{-1}) dose of imidacloprid. The loss of cyclic characters of sexual activity indicated the disturbed endocrinology of reproduction (Bretveld *et al*., 2006). Borgeest *et al*. (2002) experienced a significant increase in the percentage of days in estrous phase compared with control and methoxychlor treated mice.

The weight of ovary decreased non-significantly in F_1 generation and significantly increased in F_2 generation (Table 1). Organophosphates like methyl parathion, dimethioate and mono-crotophos given to female albino rats have also resulted in significant decrease in the ovarian weights (Kaur and Dhanju, 2005). Reduction in ovarian weight observed in rats exposed to imidacloprid at high dose levels may be due to significant pathomorphological changes. Similar results have been reported in rats treated with monocrotophos and also suggested that decrease in weight of ovary was due to extensive fibrosis and atretic follicles (Radhika and Kaliwal, 2001).

Table 1: Relative organ weight (g) of female rats in F_1 and F_2 generation orally administered with imidacloprid (mg kg^{-1} bw day^{-1})

Organs	F_1			F_2		
	0 mg	10 mg	20 mg	0 mg	10 mg	20 mg
Ovary	0.04 ± 0.001	0.030±0.004	0.03 ± 0.001	0.020±0.002	0.030±0.004	0.030±0.001**
Oviduct	0.03 ± 0.007	0.018±0.002	0.02 ± 0.004	0.013±0.001	0.009±0.001	0.011±0.001
Uterus	0.14 ± 0.020	0.210±0.043	0.19 ± 0.015	0.060±0.019	0.070±0.010	0.098±0.005*
Vagina	0.04 ± 0.002	0.070±0.010*	0.05±0.009**	0.040±0.002	0.068±0.01*	0.05±0.009**

Values represent the mean ± SE of 6 animals in each group; *Significantly different from control at P<0.05; **Significantly different from control at P<0.01

The phosphatases (ACP - acid phosphatase and AKP - alkaline phosphatase) in ovaries increased significantly at higher dose level in F_1 and F_2 generation (Table 2). Probably the increase in ALP level occurred due to the damage of the cells in liver, kidney, small intestine and bone resulting in the liberation of this enzyme in blood systems (Zimmerman, 1969).

Histologically, the sections of ovary of control rats showed different stages of follicles and sections of higher dose of treated rats showed more number of atreric follicles as compared to control rats. All the stages of follicular development *viz.*, primary, secondary, tertiary, early antral and antral were observed in control and imidacloprid treated rats in F_1 and F_2 generation. (Fig. 1 and 2) The total number of normal follicles did not differ significantly in control and imidacloprid treated ovaries. However, percentage atresia of follicles was found higher in treated rat ovaries as compared to their respective control rats (Table 3). Imidacloprid might have exerted some deleterious effects on ovaries in terms of increased atresia at early antral stages of ovarian follicles either by directly targeting the tissues or indirectly via alterations of endogenous hormones (Borgest *et al*., 2002, Bretveld *et al*., 2006). Repeated administration of cypermethrin in rats also resulted in loss of follicular cells and oocytes in ovaries (Grewal *et al*., 2010).

Table 2: Ovary acid phosphatase (ACP) and alkaline phosphatase(AKP) [µmole g^{-1}] of female rats in F_1 and F_2 generation orally administered with imidacloprid (mg kg^{-1} bw day^{-1})

Parameters	F_1			F_2		
	0 mg	10 mg	20 mg	0 mg	10 mg	20 mg
AKP	1.32±0.29	3.84±0.49	4.00±4.02	1.25±0.16	4.02±0.51*	4.40±0.41*
ACP	14.30±1.70	17.72±0.60***	22.89±3.74***	13.87±1.54	18.27±0.36***	23.5±3.50***

Values represent the mean ± SE of 6 animals in each group;
*Significantly different from control at $P_{<0.05}$; ***Significantly different from control at $P_{<0.001}$

Table 3: Effect of imidacloprid on percentage atresia in ovaries of F_1 and F_2 generation female rats

Treatments	Normal follicles (No.)	Atretic follicles (No.)	Total follicles (No.)	Atresia (%)
Control				
F_1	33±0.70	22±0.02	60±0.78	36.6±0.42
F_2	32±2.17	22±2.62	59±2.17	36.0±0.46
10 mg imidacloprid kg^{-1} bw day^{-1}				
F_1	30±2.82	13±5.65	43±2.82	30.23±2.11*
F_2	26±2.65	21±1.87	47±10.9	44.68±10.7
20 mg imidacloprid kg^{-1} bw day^{-1}				
F_1	27±4.24	25±6.36	52±2.12	48.07±2.12
F_2	33±0.76	16±1.87	49±2.14	32.65±7.60*

Values represent the mean ± SE of 6 animals in each group
*Significantly different from control at $P_{<0.05}$

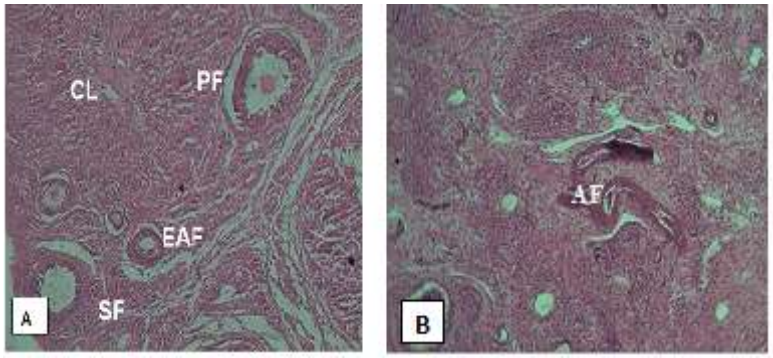

Fig. 1: A) TS of ovary of control rat of F_1 generation showing different stages of follicles i.e. primary follicles (PF), secondary follicles (SF), tertiary follicles (TF), early antral follicles (EAF), antral follicle (AF) and corpus luteum (CL) (HE x100); B) T.S of ovary of T_2 rat showing atretic primary, secondary and tertairy follicles

The follicular kinetics in serial sections of the ovaries of control, lower and higher dose of imidacloprid-treated rats were studied under light microscope. The follicles were categorized into six groups i.e. primordial, primary, secondary, tertiary, early antral and antral follicles and corpus luteum. It was observed that the number of normal follicles in all the stages of follicles was higher in control group and their number decreased in the ovaries of rats treated with imidacloprid in F_1 and F_2 generation.

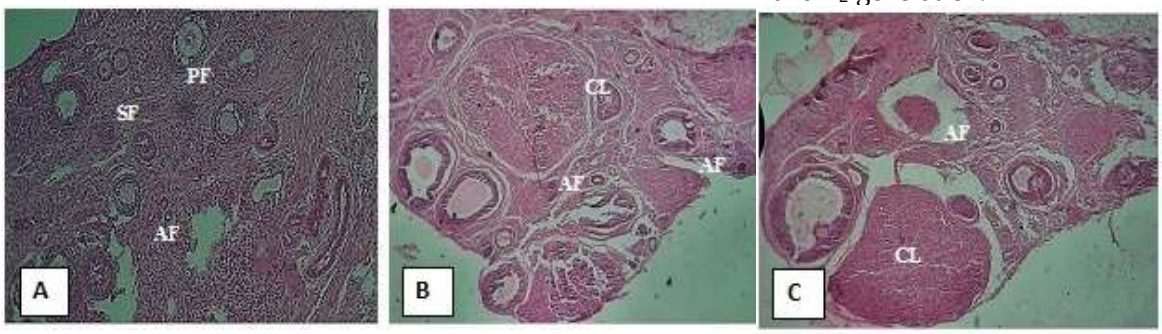

Fig. 2: A) TS of ovary of control rat of F_2 generation showing different stages of follicles i.e. primary follicles (PF), secondary follicles (SF), tertiary follicles (TF), early antral follicles (EAF), antral follicle (AF) and corpus luteum (CL) (HE x100); B) TS of ovary of rats treated with 10 mg imidacloprid kg^{-1} bw day^{-1} showing tertiaty, early antral and antral atretic follicles; C) TS of ovary of rats treated with 20 mg imidacloprid kg^{-1} bw day^{-1} showing atretic primary, secondary and tertairy follicles and corpus luteum

Atresia is the physiological mode of disposing of follicles in the ovary and the prevalence of oxidative stress in rats would lead to increased atresia. This is substantiated by the work of Gupta *et*

al. (2006). Kapoor *et al*. (2011) reported that ovary section of rats showed the presence of cytoplasmic clumping of oocytes which created a space between cytoplasm and zona pellucida in most of follicles of ovary of rats exposed to 20 mg kg^{-1} day^{-1} imidacloprid. Guney *et al*. (2007a,b) also obtained similar results in ovary of rats after exposure of methidathion.

It may be concluded that higher dose of imidacloprid (20 mg kg^{-1} day^{-1}) causes significant reproductive alterations in the female rats of F_1 and F_2 generation. Hence 10 mg kg^{-1} day^{-1} may be considered as NOEL (no observed effect level) dose for two generation female reproductive toxicity through histopathological and reproductive behaviour in female rats.

Acknowledgements: We are thankful to the Head, Department of Zoology, PAU, Ludhiana, Punjab (India) for providing facilities for conducting research.

Ethical statement: The present study was conducted in compliance with the institutional ethical guidelines and after obtaining approval from the concerned bodies.

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