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Original Article

A Three Generation Study with Effect of Imidacloprid in Rats: Biochemical and Histopathological Investigation

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ABSTRACT

Objectives: This study was designed to evaluate the dose-dependent toxic effects of imidacloprid on the female rats that were treated through three generations (F0, F1, and F2). F2 female rats were sacrificed at the end of the experiment to see the long-term effect of imidacloprid. **Materials and Methods:** Rats were divided into three groups of 6 each. Group I served as control. Group II served as treated I and given 1/45th LD_{50} (10 mg/kg/day) of imidacloprid. Group III served as treated II and given 1/22th LD_{50} (20 mg/kg/day) of imidacloprid. After 60 days, oral administration of imidacloprid females were mated with normal males to get F1 and F2 generation. F2 generation female rats were sacrificed at the end of the experiment. Biochemical and a histopathological investigation was done for three groups of F2 generation and statistically analyzed by ANOVA. **Results:** Average feed intake of F2 female rats was significantly reduced ($P < 0.01$) at 20 mg/kg/day dose of imidacloprid. There was a significant increase in the activity of alanine aminotransferase, AKP, and glucose 6-phosphate dehydrogenase in Group III rats of F2 generation. There was a significant decrease in acetylcholine esterase activity in plasma and brain of both the imidacloprid treated groups. Tissue samples of liver, kidney, and brain of females of F2 generation showed histopathological condition. **Conclusion:** The results indicated that imidacloprid at a dose of 20 mg/kg bw/day exerts significant toxicological effects on biochemical and histological studies of F2 generation females as compare to 10 mg/kg bw/day.

Key words: Biochemical analysis, histopathology, imidacloprid, wistar albino rat

INTRODUCTION

Imidacloprid is a chlorinated analog of nicotine, which belongs to the class of neonicotinoid insecticides. It has low vapor pressure, and the technical product (94.0% IM) has a moderate order of toxicity with respect to ingestion in the rat, but appears to be less toxic when absorbed by the skin or inhaled. Imidacloprid is a neonicotinoid insecticide and classified under toxicity Class II/III agents by United States Environmental Protection Agency.[1] It acts as an agonist at the postsynaptic nicotinic acetylcholine (ACh) receptor in insects.[2] Human exposure to imidacloprid, especially pregnant women is relatively possible due to its extensive use as an insecticide, with low soil persistence

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and high insecticidal activity at low application rates. Toxicological studies of imidacloprid are limited, and the acceptable daily intake was reported as 0.006 mg/kg/day based on the majority of unpublished reports.[3] In one study, pregnant rats were fed technical grade imidacloprid throughout pregnancy and lactation at doses of 0, 100, 250, and 750 ppm.[4] Imidacloprid was not found to affect reproductive variables or cause birth defects. Maternal toxicity of pesticides on experimental animals was studied by many previous investigators like Becker *et al*., 1988[5] reported that, dose-related reductions in body weight gain were observed in pregnant rats and rabbits at 30 and 100 mg/kg bw/day and at doses <24 mg/kg bw/day in pregnant female rabbits when imidacloprid was daily administered by gavage to mated female wistar rats and chinchilla rabbits at a dose of 0, 10, 30 or 100 mg and 0, 8, 24 or 72 mg respectively, on days of organogenesis period. Changes in the biochemical profile indicate alterations in the metabolism of the organism resulting from the effect of the pesticide and they make it possible to study the mechanisms of the effects of these pesticides.[6] The liver is rich source of aminotransferases (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]) and alkaline phosphatase (ALP). The hepatic injury is accompanied by hepatic inflammation or necrosis (death of cells). When the hepatic injury is produced, the hepatocytic enzymes are released into the circulation.[7]

Hence, the present study was aimed to evaluate the effect of two doses of imidacloprid on biochemical and histopathology of females of F2 generation to see the long term effect on the third generation.

MATERIALS AND METHODS

Eighteen were female and 18 were male wistar albino rats, obtained from Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The animals were housed in groups of two rats per cage. The rats were acclimatized for 1-week before using them for experimentation. The rats were maintained under controlled conditions of temperature (22°C ± 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.

Eighteen female albino rats (6 rats/each group) were divided into three groups. The first group (control group) received corn oil orally. The second group received 1/45th of LD_{50} of imidacloprid, and the third group received 1/22th of LD_{50} of imidacloprid. Adequate dilutions were made with corn oil to achieve the test concentrations. The test concentrations were calculated from the percentage of active ingredients in commercial formulations. After treatment for 2 months, these 18 females were then mated with 18 male rats (one male for one female rat) for 2 weeks. Vaginal smears were examined and the day when sperm was detected was considered to be the first day of pregnancy. The pregnant rats (F0) were treated with 1/45th and 1/22th LD_{50} of imidacloprid. Dams and their offsprings were treated with imidacloprid during the periods of mating, gestation, and lactation. The offsprings of different dams in a group of each generation were mated among themselves through three generations. F1 and F2 generations were attained by the same procedure.

All F2 rats were weighed and sacrificed by chloroform anesthesia at the end of the experiment. The tissues of liver, kidney, and brain were collected in 10% neutral formalin. After washing in running water and dehydration in alcohol, tissues were embedded and 5 μm paraffin sections cut and stained with hematoxylin and eosin and examined under light microscope.

After sacrificing, blood samples were collected directly from the heart of F2 rats, drawn into heparinized tubes and plasma was separated by centrifuging at 3000 rpm for 10 min at room temperature. The effect of imidacloprid on AST, ALT was estimated by the method of Reitman and Frankel as described by Bergmeyer[8] and acid phosphatase (ACP), ALP was estimated by method of Bessey *et al*.[9] glucose 6-phosphate dehydrogenase by the method of Deutsch,[10] ACh esterase (AChE) activity by the method of Voss and Sachsse[11] and total protein by the method of Lowry *et al*.[12]

Statistical methods

Biochemical analyses were presented as the mean ± standard error of means. Comparisons were made between control and treated groups using "Analysis of Variance (ANOVA)" as a Statgraphics Statistical Package.

RESULTS

Clinical signs

The most consistent finding was aggression/hyperactivity, which was observed throughout the study F2 females of imidacloprid treated groups. Some females of the low and high dose groups had vaginal discharges. Mean estrous cycle days were significantly reduced in a higher dose (T2) treated group of F0 females (4.06 ± 0.03, $P < 0.01$) as compared to control (5 ± 0.21).

Clinical observations

No signs of adverse effects were seen in the clinical appearance of newborn in F1 and F2 generations. The number of offsprings in F1 and F2 generations are shown

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in Table 1. The final body weights of F2 female rats are given in Table 2. There was no significant disruption in the cyclicity of female rats.

Body and relative organ weight

In the present study, a significant decrease in body weight of Group III rats was observed in comparison to control. There was no significant change in the relative organ weight of imidacloprid treated female rats of F2 generation as compared to control at ($P < 0.05$) as shown in Table 3. The weight of liver increased nonsignificantly in all the three groups of F2 generation.

The weight of endocrine glands showed no significant change in all the treated groups of F2 generation as shown in Table 4. The weight of adrenal increased significantly in a lower dose of imidacloprid treated group.

Table 1: Number of male and female offsprings in groups I-III for three generations

Groups	F0	F1			F2		
	Female	Male offsprings	Female offsprings	Total	Male offsprings	Female offsprings	Total
I	6	20	16	36	25	17	42
II	6	18	7	25	19	17	36
III	6	20	13	33	27	10	37

n=6 animals in each group

Table 2: Effect of imidacloprid on body weight of female albino rats of F2 generation as compared to control

Weeks	F2		
	Control	Imidacloprid 1/45th of LD_{50}	Imidacloprid 1/22th of LD_{50}
I	71.7±4.0	70.0±2.2	66.6±2.5
II	77.5±3.6	75.0±2.2	71.6±2.5
III	82.5±3.6	79.1±2.7	77.5±2.1
IV	88.3±3.3	85.0±2.9	74.0±2.3*

Values represent the mean±SE of 6 animals in each group, *Significantly different from control at *P*<0.05. SE=Standard error

Table 3: Relative organ weight (g) data of imidacloprid treated female rats of F2 generation as compared to control

Organs	Control	Imidacloprid 1/45th of LD_{50}	Imidacloprid 1/22th of LD_{50}
Brain	0.89±0.13	0.83±0.16	0.79±0.03
Liver	3.95±0.17	4.05±0.16	4.11±0.15
Stomach	1.66±0.14	2.00±0.26	2.11±0.26
Lungs	0.53±0.06	0.62±0.00	0.68±0.03
Heart	0.31±0.01	0.31±0.00	0.32±0.00
Spleen	0.24±0.01	0.25±0.00	0.25±0.01**
Kidney	0.67±0.01	0.71±0.01	0.77±0.02

Values represent the mean±SE of 6 animals in each group. * This is written by mistake. There is no citation available. **Significantly different from control at *P*<0.01. SE=Standard error

Biochemical parameters

There was a significant increase in activities of ALT, ACP, and AKP enzymes and a nonsignificant increase in AST enzyme level as shown in Table 5.

In the present study, the brain and plasma AChE activity was significantly reduced at higher dose level as shown in Table 6.

Histology

A liver section of control rats showed radially arranged hepatic cords around the central vein [Figure 1]. Liver sections of 1/45th LD_{50} of imidacloprid treated rats of F2 generation revealed dilation of the central vein and dilation of sinusoids and infiltration of leukocytes. Liver sections of 1/22th LD_{50} of imidacloprid treated rats revealed dilation of the central vein, infiltration by a large mass of leukocytic inflammatory cells and many pyknotic nuclei were also observed in (1/22th of LD50) imidacloprid treated rats.

Brain sections of female rats are shown in Figure 2. Brain sections of control rat showed the normal structure

Table 4: Relative organ weight (g) data of endocrine glands of female rats of F2 generation

Organs	Control	Imidacloprid 1/45th of LD_{50}	Imidacloprid 1/22th of LD_{50}
Adrenal	0.02±0.001	0.03±0.006*	0.018±0.002
Thyroid	0.24±0.020	0.23±0.016	0.209±0.007
Parathyroid	0.08±0.010	0.09±0.010	0.09±0.008

Values represent the mean±SE of 6 animals in each group. *Significantly different from control at *P*<0.05. SE=Standard error

Table 5: Plasma biochemical data of imidacloprid treated female rats of F2 generation

Parameters	Dosage/mg/kg/d 0 (control)	Imidacloprid 1/45th of LD_{50}	Imidacloprid 1/22th of LD_{50}
ALT (U/L)	32.27±4.70	39.78±3.64	45.77±1.92*
AST (U/L)	119.27±29.40	136.20±22.97	149.91±12.13
AKP (U/L)	85.18±18.49	113.10±19.10	144.31±17.49*
ACP (U/L)	1.38±0.25	1.37±0.23	2.40±0.39
Total proteins (g/dl)	7.05±1.13	7.51±0.58	7.89±0.77
G6PD (U/L)	1061±56.89	1214±61.92	2186±869.50*

Values represent the mean±SE of 6 animals in each group. *Significantly different from control at *P*<0.05. ALT=Alanine aminotransferase, AST=Aspartate aminotransferase, ACP=Acid phosphatase, G6PD=Glucose 6-phosphate dehydrogenase, SE=Standard error

Table 6: AChE inhibition of brain and plasma of female rats of F2 generation

Dosage (mg/kg/day)	AChE (U/L)	
	Plasma	Brain
Control	912.9±94.37	846.77±206.43
Imidacloprid 1/45th of LD_{50}	763.5±92.64	720.33±240.19
Imidacloprid 1/22th of LD_{50}	604.3±40.51*	613.6±92.62*

Values represent the mean±SE of 6 animals in each group, *Significantly different from control at *P*<0.05. AChE=Acetyl cholinesterase, SE=Standard error

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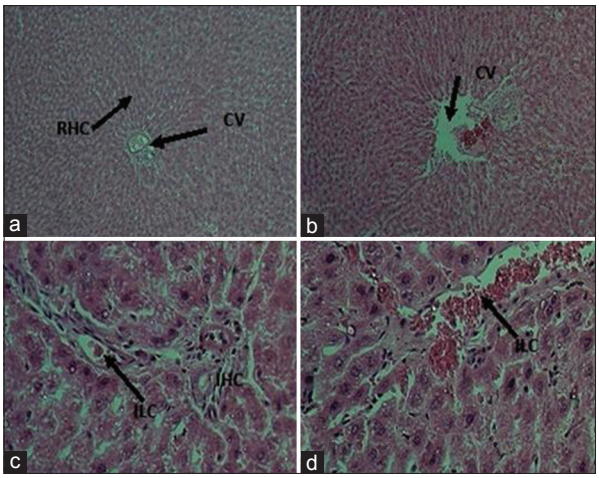


Figure 1: (a) T.S of liver of control rat of F2 generation showing radially arranged hepatic cords (RHC) around the central vein (×100) (b) T.S of liver showing dilation of central vein (H and E × 100) (c) T.S of liver of treated rat showing pyknotic nuclei and irregularly arranged hepatic cords (IHC) and infilteration of leukocyte cells in central vein (H and E × 400) (d) T.S of liver of treated rat showing infilteration by large mass of leukocytic inflammatory cells (H and E × 400)

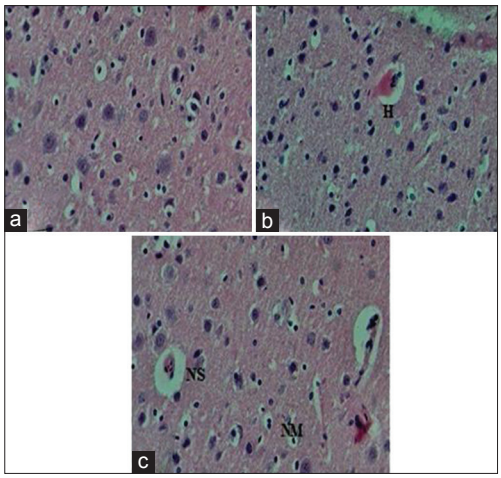


Figure 2: (a) T.S of brain of control rat of F2 generation showing normal structure of neurons and granular layers (H and E × 400) (b) T.S of brain of T1 rat showing perivascular hemorrhage and degeneration of neurons (H and E × 400) (c) T.S of brain of T2 rat showing neuronal swelling (NS) and nuclear migration (NM) (H and E × 400)

of neurons and granular layers. The brain of T1 rat showed perivascular hemorrhage and degeneration of neurons. Brain sections of rats treated with $1/22^{th}$ LD_{50} of imidacloprid showed perivascular hemorrhage and nuclear migration of neurons.

Kidney section of control female rats showed well-demarcated cortex with an intact capsule with well-formed glomerular tuft and tubules as shown in Figure 3. The histopathological changes in rats treated with imidacloprid showed enlargement in a parietal layer of Bowman's capsule and degeneration of tubules and lobulation in the glomeruli in lower and higher dose treated rats. The data of the present study have indicated that imidacloprid have induced toxicological effects to female rats at 20 mg/kg/day dose level.

DISCUSSION

In the present study, a significant decrease in body weight of Group III rats was observed in comparison to control. A similar decrease in body weight was observed in 90 days oral toxicity study of imidacloprid in female rats with doses of 0, 5, 10, and 20 mg/kg/day. Decrease in the body weight gain was observed at 20 mg/kg/day and at necropsy the relative body weights of liver, kidney, spleen, and adrenal was also significantly increased at this dose level.[13]

However, the nonsignificant increase in weight of the liver was found to be associated with a concomitant increase in the activity of serum AST and ALT. It is important to note that the elevated activity of serum AST and ALT recorded in the study may be due to tissue of liver enzyme loss. This has been confirmed by hepatocellular damage in the higher dose treated animals. Our present knowledge of liver changes induced by imidacloprid is both limited and equivocal.[14]

The weight of parathyroid increased nonsignificantly in lower and higher dose of imidacloprid treated groups. No data were found evaluating the potential of imidacloprid to disrupt endocrine function. Imidacloprid is included in the draft list of initial chemicals for screening under the U.S. EPA Endocrine Disruptor Screening Program.[15] Increased adrenal weight might reflect a state of physiological stress in the body of rats. Glucocorticoids released from adrenals might play an important role in the mediation of depressed immune response. It was reported that rats exposed to liquid mosquito repellent containing allethrin (3.6% w/w) also had a significant increase in relative weights of adrenal glands in the male rats.[16]

Freedland and Kramer[17] suggested that enzyme levels are sensitive indicators of tissue damage, since they are liberated from cells even when the magnitude of lesions is not sufficient for morphological detection. This was confirmed by the result obtained in our study. Furthermore, it was reported that ALT level elevation is due to the leakage of damaged membranes.[18]

In the present study, the brain and plasma AChE activity was significantly reduced at higher dose level as shown in Table 6. According to one study, the cause of AChE inhibition is unknown because imidacloprid is not ChE inhibitor, since plasma AChE is synthesized in the liver, the decrease in plasma AChE activity may be related to observed changes in liver function. Earlier studies have shown that the concentration of pesticides and metabolites in plasma and brain generally correlate with the severity of toxicity and

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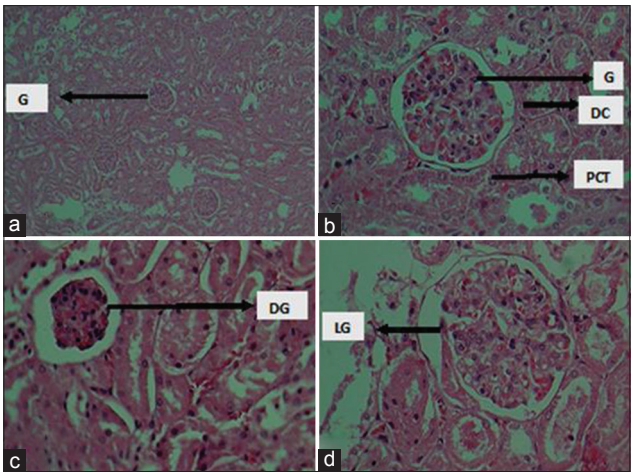


Figure 3: (a) T.S of kidney of control rat of F2 generation showing intact structures of the glomeruli (g) and renal tubules (H and E ×100) (b) Magnified view of kidney of control rat showing intact glomeruli (g) (H and E ×400) (c) T.S of kidney of T1 rat showing glomerular degenerations (DG) and enlargement in parietal layer of Bowman's capsules (H and E ×400) (d) T.S of kidney of T2 rat showing tubular degenerations and lobulation in the glomeruli (LG) (H and E ×400)

symptoms of neurotoxicity, which were found to increase with the pesticide concentration in the brain.

The liver is the center for detoxifying any foreign compounds entering the body. Hence, it uniquely exposed to a wide variety of exogenous and endogenous products. These include environmental toxins and chemicals present in food or drinking.[19] Mohany *et al*. reported that animals treated with 0.21 mg/kg IMI for 4 weeks showed heavily congested central vein and blood sinusoids, widely distributed pyknotic nuclei, and leukocyte infiltration in rat liver.[20] IMI at 1/10th of LD50 treatment resulted in dilatations of central vein and sinusoids between hepatocytes.[21] High dose of IMI (20 mg/kg/day) resulted in mild focal necrosis with swollen cellular nuclei and cytoplasmic lesions in rat liver and slight degeneration of tubules and glomeruli of kidney of the female rats.[13] IMI produced similar histopathological lesions in the liver, kidneys, and brain of Japanese quail exposed to chemical for 6 weeks[22] and in layer chickens exposed to 139 mg/kg IMI.[23] Hepatocellular hypertrophy and fatty changes in the liver were also observed in a 3 weeks study conducted with thiacloprid in mice.[24] The study suggested that toxic responses occur relatively frequently in the liver compared with other organs mainly because the liver is a predominant organ for the metabolism, and is also the first major organ to be exposed to ingested toxins, due to its portal blood supply.[25]

It was reported that, brain sections of rats treated with 80 mg/kg body weight revealed marked congestion in cerebellum, degeneration of Purkinje cells with loss of dendrites, vacuolation around neurons, and shrunken neurons on 14th day of experiment.[26]

On day 28, brain sections revealed vacuolation around the neuronal cell body, chromatolysis, and marked congestion. Bhardwaj *et al*.[13] also reported brain damage showing degenerative changes in Purkinje cells and loss of granules in the granular layer. Necrosed Purkinje cells and loss of granules in the granular layer of the cerebellum have also provided support to the neurobehavioral effects indicating accumulation of imidacloprid and its metabolites in the brain.

Bhardwaj *et al*.[13] reported kidney damage including degeneration of tubular and glomerular structures after oral administration of rats with 20 mg/kg/d imidacloprid. Imidacloprid at oral doses of 15 mg/kg/day produced apparent histopathological changes in liver, kidneys, heart, and brain in male and female mice on 28th day of insecticide treatment. Vacuole degenerations, dilatation of sinusoids, dissociated remark cordons, pyknotic nuclei, and leukocyte infiltration were observed in livers of male and female mice. There was shrinkage of glomeruli and degeneration of epithelial cells in kidneys, degeneration in hearts, and focal gliosis in brains of male and female mice.[27]

CONCLUSION

Although the results obtained from this study showed histopathological and biochemical effects in rats fed with imidacloprid at two doses of imidacloprid. The results were severe at a higher dose (20 mg/kg bw/day) in comparison to lower dose (10 mg/kg bw/day). Long-term consumption of imidacloprid throughout three generations did not cause severe health concerns on rats. Therefore, long-term studies with imidacloprid should be performed on other species of rats.

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Conflicts of interest

There are no conflicts of interest.

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