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Effect of imidacloprid on plasma and tissue biochemistry of albino rat

KEYWORDS: Imidacloprid, alanine aminotransferase (ALT), aspartate aminotransferase (AST) acid phosphatase (ACP)

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ABSTRACT *Imidacloprid was given orally to female albino rats for 60 days. Rats were divided into three groups. The control animals (first group) were given corn oil. Second group treated with 1/45th LD_{50} of imidacloprid and third group with 1/22th LD_{50} of imidacloprid to assess the alterations in the level of some biochemical prameters in tissues. Liver alanine aminotransferase (ALT), aspartate aminotransferase (AST) increased significantly at high dose of imidacloprid. Acid phosphatase (ACP) and alkaline phosphatase (AKP) activity increased significantly at 1/22th LD_{50} of imidacloprid in liver and ovary. Significant decrease was observed in plasma 3β- hydroxysteroid dehydrogenase (3β HSDH) activity at 1/22th LD_{50} of imidacloprid. Histological examination of ovary of 1/22th LD_{50} of imidacloprid showed atretic follicles. Imidacloprid at 1/22th LD_{50} was found to be toxic to rats.*

Introduction

Imidacloprid, a relatively new, systemic insecticide related to nicotine, was introduced to the market in 1991 as the first chloronicotinyl insecticide and has become largest selling insecticide worldwide (Menscke, 2002). In agriculture, it is most commonly used on rice, cereal, maize, potatoes, vegetables, sugar beets, fruits, cotton and hops for control of sucking insects, coleopteran and others (Cox, 2001). Imidacloprid and its analogs are remarkably potent neurotoxic insecticides, which act as nicotinic acetylcholine receptor (nAChRs) agonists (Matsuda, 2005). nAChRs play a central role in rapid cholinergic synaptic transmission and are important targets of insecticides (Sattelle, 1990). It is moderately toxic and its acute oral LD_{50} is 450 mg/kg for rats and 150 mg/kg for mice (Tomlin, 1997). Recently imidacloprid has raised concern because of reports of egg shell thinning; reduced egg production and hatching time which are considered as signs of possible endocrine disruption (Berny et al., 1999 and Matsuda et al., 2001).

Aminotransferases and phosphatases are important and critical enzymes in the liver metabolic activity and are responsible for detoxificiation processes. So any interference in various enzyme levels lead to biochemical impairment and lesions of the tissue. The liver is the principal target of imidacloprid toxicity, as demonstrated by its elevated serum transaminase, alkaline phosphatase and/or glutamate dehydrogenase activities; and alterations of other clinical parameters(Rahman et al.,2000). Many pesticides having endocrine disruptors properties are known to adversely impair reproductive competence of male and females (Yousef, 2010). Although it is widely used worldwide, there is still less work done related to its toxicity in female rats. Therefore, the present study has been designed to evaluate the effect of imidacloprid on transaminases and phosphatases in female albino rats.

Materials and methods

Commercial product of imidacloprid (Confidor, 17.8%, SL) used in this study was purchased from the local market in Ludhiana, India. The study was conducted on sexually mature female Wistar albino rats, 3 months of age, weighing 100–150 g obtained from Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana. 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^{0}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 (IAEC) approved this experimental protocol. Adult females were divided into three groups. Group 1 served as Control and given corn oil through oral intubation. Group 2 served as Treated 1 given 1/45th LD_{50} of imidacloprid. Group 3 served as Treated 2 given 1/22th LD_{50} of imidacloprid for 60 days.

Biochemical Measurements

After completion of 60 days rats were sacrificed, blood samples were drawn into heparinized tubes and plasma was separated by centrifuging at 3000 rpm for 10 minutes at room temperature. The tissue sample of ovary was homogenized in the phosphate buffer saline. The activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) were estimated by the method of Reitman and Frankel as described by Bergmeyer (1974) and acid phosphatase (ACP), alkaline phosphatase (ALP) was estimated by method of Bessey et al (1946). The enzyme 3 β-hydroxy steroid dehydrogenase was estimated in ovary and plasma by the method of Angular et al 1992. Lactate dehydrogenase (LDH) was estimated by method of King (1965) and urea by method of Hawk (1968) and total proteins by Lowry et al (1951). Representative samples from the ovaries 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 haematoxylin and eosin as per the method of Luna (1968). Biochemical analyses were presented as the mean ± standard error of means (S.E.M). Comparisons were made between control and treated groups using ''Analysis of Variance (ANOVA)'' as a statgraphics statistical package.

Results and Discussion

Daily oral administration of imidacloprid at 1/45th LD_{50} imidacloprid and 1/22th LD_{50} of imidacloprid produced a significant rise in the levels of ALT, AST, ALP and ACP in liver and ovary tissue. Table 1 shows the relative organ weight data of female rats orally administered imidacloprid. There was non-significant decrease in weight of oviduct. Table 2 shows that there was significant increase in ALT and AST activity in the liver. Bhardwaj et al (2010) also observed that oral adminis-

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tration of imidacloprid in female rats at the rate of 5,10 and 20 mg/kgbw/day for 90 days resulted in elevation of serum ALT, AST, glucose, blood urea nitrogen (BUN). There was significant increase in ALP and ACP activity of liver and ovary tissue as shown in Table 3. It has been suggested that an increase in alkaline phosphatase (ALP) level occurs due to the damage of the cells of liver, kidney, small intestine, and bone resulting in the liberation of this enzyme in the blood systems (Zimmerman, 1969). Balani et al (2011) evaluated the toxic effects of oral administration of imidacloprid in male white leg horn chicken at 1.25, 1.67 and 2.5 mg/kgbw for 28 days and noticed an increase in ALT, no change in AST, serum total protein, total globulins, total albumin and serum creatinine levels.

Table 3 showed that there was significant increase in the proteins of ovary and liver tissue in the present study. The elevation of proteins is reported to occur in conditions when the cells are subjected to wide variety of environmental assaults including toxins, poisons and pollutants and is mainly due to stimulation of the synthesis of acute phase protein and corresponding m-RNA (Puga,1974), which buffer them from harm (Welch, 1993). Elevation of proteins might also be due to destruction of tissues, which causes release of proteins.

3β-HSDH is an enzyme that catalyses the synthesis of progesterone from pregnenolone. There was significant decease in the activity of 3 β-hydroxysteroid dehydrogenase activity in plasma and it decreased non-significantly in ovary as shown in Table 2. Decrease in 3β-HSDH activity in treated rat ovary indicates altered levels of reproductive hormones in rats (Bretveld et al., 2006). The effect was also observed in the histology of ovarian follicles. Fig 1 shows that ovary of control rat showed normal primary and secondary follicles and imidacloprid (1/22th LD_{50}) treated ovary sections showed developed and atretic follicles. 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 1/22th LD_{50} of imidacloprid imidacloprid is in agreement of work of Guney et al; (2007a,b) who has shown similar results in ovary of rats after exposure of methidathion.

Table 2 showed that there was non-significant decrease in the LDH enzyme activity in plasma. LDH is an important glycolytic enzyme in biochemical systems and is inducible by oxygen stress (Shekari et al., 2008). Several reports have revealed decreased LDH activity in tissues under various pesticide toxicity conditions (Tripathi, 1991; Mishra, 2003). This might be due to the higher glycolysis rate, which is the only energy producing pathway for the animal when it is under stress conditions. Non-significant increase was observed in plasma urea level (Table 2). The increase in urea is probably due to altered metabolic pathway after treatment that prevented the natural excretion of urea from body (Etebari et al., 2004).

Conclusion

Imidacloprid at 1/22th LD_{50} of imidacloprid exposure leads to marked alterations in plasma and tissue biochemistry. So, imidacloprid at 1/22th LD_{50} was found to be potent toxic agent affecting metabolism and reproduction in treated rats.

Table 1

Relative organ weight (g %) data of organs of female rats orally administered imidacloprid

Organs	Control	Imidacloprid 1/45th of LD_{50}	Imidacloprid 1/22th of LD_{50}
Net body weight Gain(g/100gbw)	45 ± 4.47	35 ± 6.32	42.5 ± 5.12
Oviduct	0.03 ± 0.022	0.01 ± 0.001	0.009 ± 0.0005
Vagina	0.05 ± 0.007	0.05 ± 0.011	0.05 ± 0.010

Values represent the mean ± SE of 6 animals in each group

Table 2

Effect of imidacloprid on plasma, ovary and liver enzyme activities

	Control	Imidacloprid 1/45th of LD_{50}	Imidacloprid 1/22th of LD_{50}
Ovary			
3βHSDH (µmole/g ovary)	9.46 ± 0.50	7.807 ± 0.43	7.917 ± 0.76
Plasma			
3 β HSDH (U/L)	18.43 ± 0.662	15.68 ± 0.563*	13.20 ± 0.602**
LDH(µ mole/ml/ min)	65.31 ± 26.11	55.85 ± 15.33	46.35 ± 11.39
Urea (mg/dl)	25.47 ± 2.635	26.33 ± 2.99	30.93 ± 1.22
Liver			
ALT (µmole/g liver)	25.88 ± 3.680	52.09 ± 3.803***	60.47 ± 7.595***
AST (µmole/g liver)	51.48 ± 4.653	60.81 ± 4.078*	69.35 ± 4.135**

Values represent the mean ± SE of 6 animals in each group

*Significantly different from control at P<0.05

**Significantly different between group at P<0.01

***Significantly different from control at P<0.001

Table 3

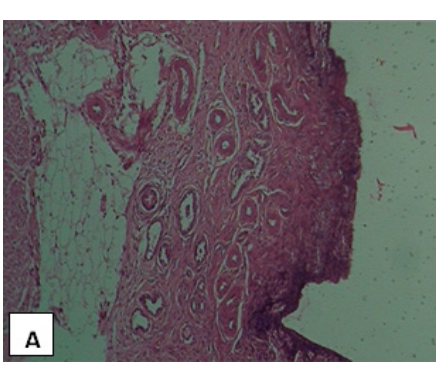
Effect of imidacloprid on tissue phosphatases and proteins

Organ	Control	Imidacloprid 1/45th of LD_{50}	Imidacloprid 1/22th of LD_{50}
ACP (µ mole/g tissue)			
Liver	26.74 ± 4.32	30.19 ± 3.23	32.58 ± 2.56
Ovary	1.048 ± 0.06	3.449 ± 0.276***	3.84 ± 0.060****
ALP (µ mole/g tissue)			
Liver	40.93 ± 0.53	44.75 ± 0.355****	45.48 ± 0.507****
Ovary	14.02 ± 1.63	16.48 ± 0.529	19.59 ± 2.74
Total proteins (mg/g)			
Liver	9.63 ± 0.744	11.960 ± 1.823	13.53 ± 0.85
Ovary	7.12 ± 0.47	10.46 ± 1.077**	11.27 ± 0.302****

**Significantly different from control at P<0.01

***Signicantly different from control at P<0.001

****Significantly different from control at P<0.0001



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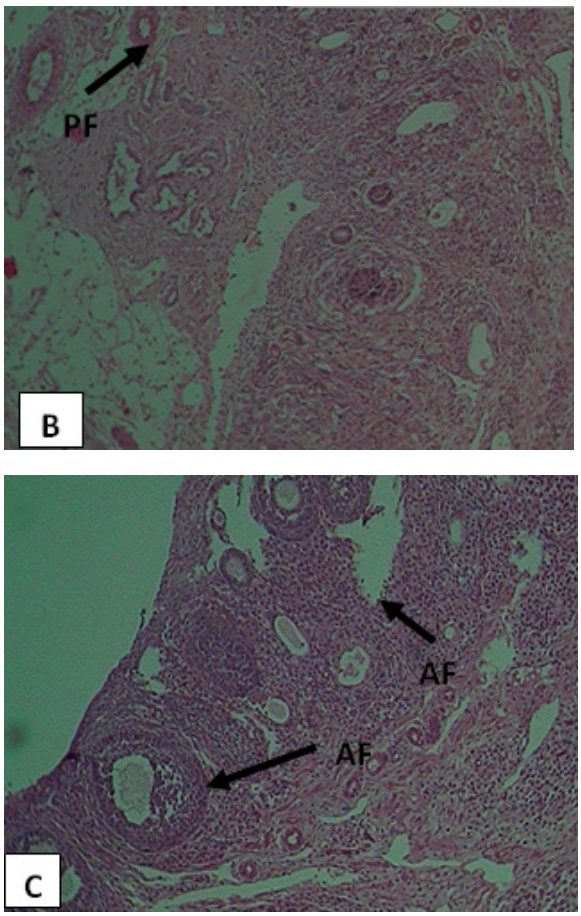

Fig 1. A. T.S of ovary of Control rats B, C. T.S of ovary of 1/45th and 1/22th LD_{50} of imidacloprid treated rats showing normal developed and atretic follicles.

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