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Imidacloprid induced histological and biochemical alterations in liver of female albino rats

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ABSTRACT

Imidacloprid is a neonicotinoid insecticide being used extensively for crop protection and pet flea control programmes. The effect of repeated oral administration of two doses of imidcloprid (1/10th and 1/50th of LD_{50} of imidacloprid) on liver of female albino rat was assessed. Histological examination of liver revealed that imidacloprid (1/10th of LD_{50}) treatment resulted in dilations of central vein and sinusoids between hepatocytes however imidacloprid (1/50th of LD_{50}) treatment did not induce histopathological changes in liver. Non significant decrease in alkaline phosphatase (AKP) activity was observed in imidacloprid treated rats. Liver aspartate aminotransferase level showed significant increase in higher dose of imidacloprid. Additionally, significant increases in plasma levels of aspatate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (AKP) were observed in the treated rats. The results suggest that the higher doses of imidacloprid at 1/10th of LD_{50} is hepatotoxic as compared to lower dose of 1/50th of LD_{50} of imidacloprid.

1. Introduction

Imidacloprid, 1[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine, a chloronicotyl, is an extensively used insecticide for crop protection world wide from the last decade due to its low soil persistence and high insecticidal activity at very low application rate [1]. It has outstanding potency and systemic action for crop protection against piercing-sucking pests and is highly effective for flea control on cats and dogs [2]. The selective toxicity of imidacloprid to insects and not to mammals is attributed to differences in the structure and binding affinity or potency at the nicotinic acetylcholine receptor [1,3]. Although previous studies have found low toxicity to mammals [4] and humans, human imidacloprid poisoning [5] and two fatal intoxication cases [6] have recently been reported. Laboratory studies of imidacloprid have shown genotoxicity in both rats [7] and cultured human lymphocytes [8]. Moreover, immunological, biochemical and neurobehavioral deficits were found in rats exposed to imidacloprid [9–12].

Aminotransferases and phosphatases are important and critical enzymes in the liver biochemical process and are responsible for detoxificiation processes [13]. 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 necrosis or hypertrophy; elevated serum transaminase, alkaline phosphatase and/or glutamate dehydrogenase activities; and alterations of other clinical parameters [10,14]. Exposure to 1/100 LD_{50} of imidacloprid also produced immunotoxicity, oxidative stress, lipid peroxidation and hepatotoxicity in male rats [12]. Imidacloprid treated birds also showed significant increase in serum aspartate aminotransferase (AST) level at 14 and 28 days of experiment, while no significant change in serum alanine aminotransferase (ALT), serum total protein, serum total albumin, serum total globulin and serum creatinine was seen [15].

Since liver is associated with metabolism and elimination of toxicants from the body and its histological and biochemical parameters are considered as key points to elucidate toxicity of the chemicals. There is paucity of information regarding the sub chronic studies of commercial imidacloprid preparation. Therefore the study has been carried out to determine changes in enzymes and histopathology in liver and plasma of imidacloprid treated rats.

2. Materials and methods

2.1. Procurement and maintenance of animals

The study was conducted on sexually mature female albino rats, 3 months of age, weighing 100–150 g obtained from Guru Angad Dev Veterinary and Animal Sciences University (GADVASU),

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Ludhiana. The animals were housed in groups of two rats per cage. The rats were acclimatized for 10 days before using them for experimentation. The rats were maintained under controlled conditions of temperature (22 ± 2 °C) and provided with 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 (VPS/2008/874-885).

2.2. Treatment with pesticide

Commercial formulation of imidacloprid (Confidor 40 SL from M/S Bayer Corporation Limited, Mumbai) was used for treatment of rats. The rats were given imidacloprid at a dose level of 45 and 9 mg/kg bw) which is 1/10th and 1/50th of LD_{50} for four weeks continuously by oral intubation. Adequate dilutions were made with vegetable oil to achieve the test concentrations. The test concentrations were calculated from the percentage of active ingredients of commercial formulations. Control rats received a similar amount of distilled water (control I)/vegetable oil (control II) orally through intubation. The body weights of control as well as treated rats was taken before the start of the treatment and then on the day of sacrifice. On the completion of experiment, the rats were sacrificed and liver was excised and weighed.

2.3. Histopathological examination

For light microscopic examination, the liver of dissected animals were cleared from adhering tissues and fixed in Bouin's fixative for 24 h and processed for paraffin embedding. After routine processing, paraffin sections of liver tissues were cut into 5–6 μm thickness and stained with haematoxylin, and eosin (H & E). Haematoxylin–eosin stained slides were studied under an optical light microscope and the architecture of hepatocytes, central vein, sinusoids and abundance of pycnotic nuclei in liver tissue was observed.

2.4. Biochemical studies

After one month of the experiment, animals of each group were anaesthetized by chloroform. Blood was collected directly from the heart in heparinized vials. Plasma was separated by centrifuging the blood at 3000 rpm at 4 °C for 15 min. The supernatant was used for estimation of aminotransferases and phosphatases. The rats were then killed by cervical dislocation and liver was dissected out, weighed and stored at −70 °C until analysis. The liver tissues (1 g) were homogenized with ultra tissue homogenizer in 5 ml of 50 mM phosphate buffer (pH 7.4). The particle free supernatant was obtained by centrifugation at 5000 rpm. for 20 min at 4 °C and used as enzyme source. The level of alkaline phosphatase (AKP) was measured in glycine buffer (0.05 mM pH 10.5) using p-nitrophenol phosphate as substrate following Bessey et al. [16] method. Aspartate aminotransferase (AST) and alanine aminotransferase activities were assayed by colorimetric method of Reitman and Frankel as described by Bergmeyer [17].

2.5. Statistical analysis

Biochemical analyses were presented as the mean ± standard error of means (S.E.M). Comparisons were made between control, vehicle and treated groups on computer using "Analysis of Variance (ANOVA)" as a Statgraphics statistical package. A "*P*" value of 0.05 was selected as a criterion for statistically significant differences.

3. Results

3.1. Body and liver weights

Mean changes in feed intake, body weight and relative liver weights are summarized in Table 1. Average feed intake was significantly reduced in female rats treated with the higher dose of the imidacloprid. It did not differ significantly in control and 1/50th of LD_{50} dose treated rats. At the end of the experiment, net body weight gain was less in treated rats as compared to control rats. The growth rate was significantly ($P < 0.005$) lower for 1/10th of LD_{50} imidacloprid than 1/50th of LD_{50} insecticide. Weight of liver significantly decreased in 1/10th of LD_{50} treated rats while nonsignificant decrease was observed with lower dose (Table 1).

3.2. Histopathological observations

Liver histological observations of control rats showed radially arranged hepatic cords around the central vein (Fig. 1A–C). However the microscopic examination of imidacloprid (1/10th and 1/50th of LD_{50}) treated rat liver revealed many dose dependent degenerative changes of variable degrees in many areas of liver. Histopathological effects of 1/10th and 1/50th of LD_{50} i.e. 45 and 9 mg/kg body weight imidacloprid on the liver of treated rats are presented in Fig. 1D. In imidacloprid (1/10th of LD_{50}) treatment, central vein and sinusoids between hepatocytes were dilated (Fig. 1D), however 1/50th of LD_{50} imidacloprid treatment did not induce significant histopathological changes in liver (Fig. 1E and F). Infiltration by large mass of leucocytic inflammatory cells and many pynotic nuclei were also observed in imidacloprid (1/10th of LD_{50}) treated rats (Fig. 1D).

3.3. Biochemical studies

Aspartate aminotransferase (AST) activity increased in plasma of both the groups of treated rats as compared to control rats (Table 2). Alanine aminotransferase and alkaline phophatase activity increased significantly in plasma of 1/10th of LD_{50} treated rats but differed non-significantly in the plasma of rats treated with lower dose of imidacloprid. Treatment of rats with imidacloprid also caused changes in the enzyme levels of aspartate aminotransferase (AST) and alkaline phosphatase (AKP) in liver tissue (Table 3). It was observed that 1/10th of LD_{50} imidacloprid caused significant increase in AST level in liver tissue (Table 3). Both the doses of imidacloprid treatment did not show any significant decrease in levels of AKP enzyme activity (Table 3).

4. Discussion

Average feed intake and net body weight gain decreased significantly in higher dose of Imidacloprid treated rats. Studies of Bhardwaj et al. [10] and Kapoor et al. [18] have also shown a significant decrease in body weight and feed intake of rats exposed to high dose of imidacloprid. The weight gain in animals serves as index of growth rate [19]. Reduced body weight gain at various doses has also been reported earlier by many workers in both the sexes of rats [20,21]. Weight of liver decreased in imidacloprid treated rats in a dose dependent manner. Loss of organ weight has been used as criterion in the assessment of drug toxicity in animals [22]. The reduced food consumption and liver weight in high dose exposed animals seems to be due to toxic potential of imidacloprid [21].

Histopathological examination of liver tissues exposed to higher dose showed dilation of central vein and blood sinusoids, wide spread pycnotic nuclei and leucocyte infilteration in hepatic tissue.