Imidacloprid induced oxidative stress and histopathological changes in liver of rats

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ABSTRACT

Imidacloprid was evaluated for its effect on oxidative stress and histopathological changes in liver of Wistar albino rats at two dose levels (19 and 38 mg/kg/day) administered orally for 10, 20 and 30 days. Effects were compared with respective control groups administered with 2% gum acacia (1ml/100g). Different parameters undertaken were liver weight, oxidative stress parameters viz. activities of superoxide dismutase (SOD), glutathione peroxidase (GPx) and levels of reduced glutathione (GSH) and malondialdehyde (MDA), cytoplasmic and membrane proteins and histopathological changes in liver. Imidacloprid at 38 mg/kg dose significantly increased (p < 0.05) organ weight and levels of MDA in 20 and 30 days group. There was significant decreased (p < 0.05) in levels of cytoplasmic and membrane proteins and activities of enzymes SOD and GPx at 38 mg/kg dose administered for 20 and 30 days. GSH levels were decreased significantly (p < 0.05) at 38 mg/kg dose administered for 30 days. Degenerative changes in hepatocytes were observed at 38 mg/kg dose administered orally for 20 and 30 days.

Key words: Female rats, Histopathology, Imidacloprid, Liver, Oxidative stress

INTRODUCTION

Imidacloprid (IMC) (1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine) was the first representative of neonicotinoid insecticides to be registered for use and at the moment the insecticide with the World’s fastest growing sales and is considered possible replacement for the widely used organophosphorus pesticide, diazinon, which is subject to phased revocation in many countries. Imidacloprid which act selectively on nicotinic acetylcholine receptors (nAChRs), is used worldwide for insect pest management and flea control in cat and dogs (Abou-Donia et al., 2008). The selective toxicity to insects over vertebrates of neonicotinoids has been shown to result, at least in part, from their selectivity to insect nAChRs (Matsuda et al., 2001; Tomizawa and Casida, 2000). Increased use of this insecticide and potential toxicity among humans warrants a heightened awareness about this compound (David et al., 2007).

Since liver is associated with metabolism of toxicants in the body and its biochemical and histopathological parameters are considered as key points to elucidate toxicity of the chemicals. There is paucity of information regarding the sub-acute studies of technical grade of imidacloprid. The present study is aimed to evaluate 30 days oral toxicity of imidacloprid to determine changes in antioxidant enzymes, biomarker of oxidative stress, LPO and histopathological changes in imidacloprid treated rats.

The results of the study may help in evaluating the safety of imidacloprid and its judicious use. The results may further be used for establishing a biomarker to imidacloprid/neonicotinoid poisoning/toxicity and thus may help in differential diagnosis of poisoning or toxicity.

MATERIALS AND METHODS

Chemicals and reagents: Imidacloprid technical grade (98% purity w/w) was obtained from Indoff Chemical Company Mumbai, India and all the chemicals used in the present experiment were procured from Analytical Rasayan and Himedia Laboratories Pvt. Ltd, India.

Experimental design: Female healthy Wistar albino rats, each weighing 120-140 g, were obtained from Small Animal House, LUVAS, Hisar. Approval from Institutional Animal Ethical Committee was obtained for use of animals. Rats were randomly divided into nine groups of six rats per group. A set of six rats was housed in a plastic cage in a temperature- and light-controlled room and the animals had free access to food and tap water. All animals were fed a commercial diet during the experiment. The nine groups of rats fell into three categories. The first category served as a control and consisted of three groups and received only 2% gum acacia prepared in distilled water for 10, 20 and 30 days respectively, by gavage. The second and third category were also made up of three groups each, the rats in the first three groups of second category treated with imidacloprid 19 mg/kg/day for 10, 20 and 30 days respectively, whereas the rats...
in the next three groups of third category received the imidacloprid at 38 mg/kg/day for 10, 20 and 30 days respectively, by gavage. All dosing was started at the same time in the morning (0800 h) to avoid the effects of biological rhythm changes.

In control, one group was randomly chosen and sacrificed, using chloroform anaesthesia, on day 10, 20 and 30 of daily administration of 2% gum acacia solution orally. Similarly, in second and third category one group was randomly chosen and sacrificed, using chloroform anaesthesia, on day 10, 20 and 30 of daily administration of imidacloprid at 19 and 38 mg/kg/day dose, respectively.

**Tissue homogenate preparation:** Liver was quickly removed, trimmed of extraneous tissue and washed with ice cold physiological saline solution and then weighed. After that liver tissue was divided into different parts. Tissue homogenate (10%) was prepared in all the biochemical parameters estimation. One part was homogenized in ice cold phosphate buffered saline for estimation of GSH, SOD, cytoplasmic and membranic proteins levels. One part of tissue was homogenized with 0.15 M KCl for estimation of GPx and remaining part was homogenized in ice cold 1.15% KCl solution for estimation of MDA.

**Biochemical parameters**

**LPO:** The formation of thiobarbituric acid-reactive substances (TBARs) in liver was monitored as an index of lipid peroxidation according to a previously described colorimetric method (Ohkhawa et al., 1972). Results are expressed as nano-moles of MDA per gram of wet tissue.

**GSH:** GSH level in tissue homogenate was measured by method of Beutler et al., (1963). The results are expressed as µmol/mg protein.

**GPx:** GPx-Px activity was measured by method of Hafeman et al., (1974) which was modification of Mills procedure. Results are expressed as U/mg protein.

**SOD:** Superoxide dismutase (SOD) activity was estimated as per the method described by Madesh and Balsubramaniam (1998). Results are expressed as U/mg protein.

**Estimation of proteins:** The levels of cytoplasmic and membranic proteins estimated in liver using Autopak kits. The soluble (cytoplasmic) proteins were estimated in the supernatant of 10% tissue homogenate and remaining pellet was washed several times with PBS at 10,000 rpm for 5 min. The supernatant was separated and pellet dissolved in PBS (7.4 pH) having 1% sodium dodecyl sulfate for 1 hour at 37°C. The solution was centrifuged at 10,000 rpm for 10 min and supernatant was used for estimation of membrane proteins.

**Histopathology:** Histopathology of liver was done according to Luna (1968). The slides were observed under microscope for assessment of histopathological changes.

**Statistical analysis:** The results are presented as mean±S.E. One way analysis of variance (ANOVA) was used for comparing data comprising of multiple treatment groups of imidacloprid as described by Panse and Sukhatme (1978). p≤0.05 was taken as the critical criterion for stastically significant differences between the data.

### RESULTS AND DISCUSSION

**Effect on liver weight:** There was significant(p<0.05) increase in the values of relative liver weight of Wistar albino rats treated with imidacloprid at 38 mg/kg dose orally daily for 20 and 30 days, respectively. (Table 1).

**Effect on LPO:** There was significant(p<0.05) increase in the values of MDA levels in tissue homogenate of liver of Wistar albino rats treated with imidacloprid at 38 mg/kg dose orally daily for 20 and 30 days, respectively. (Table 2).

**Effect on GSH:** A significant (p<0.05) decrease in the value of reduced glutathione level in tissue homogenate of liver of Wistar albino rats treated with imidacloprid at 38 mg/kg dose orally daily for 30 days was observed. (Table 2).

### Table 1: Effect of toxicity of imidacloprid on relative weight of liver (g/100g) of Wistar albino rats.

<table>
<thead>
<tr>
<th>Treatment (mg/kg,p.o.)</th>
<th>Liver weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Control (200)</td>
<td>3.05±0.009</td>
</tr>
<tr>
<td>IMI (19)</td>
<td>3.03±0.007</td>
</tr>
<tr>
<td>IMI (38)</td>
<td>3.06±0.010</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. (n=6) in each group. * Significant at level of p < 0.05.

### Table 2: Effect of toxicity of imidacloprid on MDA (nmol/g tissue) and GSH (µmol/mg protein) levels in liver of Wistar albino rats.

<table>
<thead>
<tr>
<th>Treatment (mg/kg,p.o.)</th>
<th>MDA</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Control (200)</td>
<td>171.74±3.31</td>
<td>172.29±3.93</td>
</tr>
<tr>
<td>IMI (19)</td>
<td>172.03±2.94</td>
<td>175.02±3.15</td>
</tr>
<tr>
<td>IMI (38)</td>
<td>174.12±2.79</td>
<td>184.14±1.65*</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. (n=6) in each group. * Significant at level of p < 0.05.
Table 3: Effect of toxicity of imidacloprid on GPx (U/mg protein) and SOD (U/mg protein) enzyme levels in liver of Wistar albino rats.

<table>
<thead>
<tr>
<th>Treatment (mg/kg,p.o.)</th>
<th>GPx Duration of treatment (days)</th>
<th>SOD Duration of treatment (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td>Control (200)</td>
<td>9.6±0.16</td>
<td>9.4±0.18</td>
</tr>
<tr>
<td>IMI (19)</td>
<td>9.4±0.27</td>
<td>8.9±0.18</td>
</tr>
<tr>
<td>IMI (38)</td>
<td>9.2±0.11</td>
<td>8.6±0.13*</td>
</tr>
</tbody>
</table>

Values are mean ± S.E. (n=6) in each group. * Significant at level of p < 0.05.

**Effect on GPx:** A significant (p<0.05) decrease in the activity of glutathione peroxidase enzyme in tissue homogenate of liver of Wistar albino rats treated with imidacloprid at 38 mg/kg dose orally daily for 20 and 30 days was observed. (Table 3).

**Effect on SOD:** A significant (p<0.05) decrease in the activity of SOD enzyme in tissue homogenate of liver of Wistar albino rats treated with imidacloprid at 38 mg/kg dose orally daily for 20 and 30 days was observed. (Table 3).

**Effect on cytoplasmic proteins:** A significant (p<0.05) decrease in the value cytoplasmic protein levels in tissue homogenates of liver of Wistar albino rats treated with imidacloprid at 38 mg/kg dose orally daily 20 and 30 days was observed (Table 4).

**Effect on membrane proteins:** A significant (p<0.05) decrease in the value of membrane protein levels in tissue homogenates of liver of Wistar albino rats treated with imidacloprid at 38 mg/kg dose orally daily for 20 and 30 days was observed (Table 4).

**Histopathological findings in liver:** Histopathological findings due to imidacloprid treatment in liver are represented in Figure 1. No pathological changes were observed in liver of female rats exposed at IMI (19) for 10, 20 and 30 days of exposure. Repeated exposure of IMI (38) for 10 days did not produce any changes in the hepatic parenchyma except for mild cellular swelling in the hepatocytes and sinusoidal dilation.

Repeated exposure of high dose of imidacloprid for 20 days produced degenerative changes in the hepatocytes characterized by cytoplasmic vacuolation and hyperchromasia of nuclear chromatin material. There was periportal reaction in the parenchyma characterized by infiltration of the neutrophils around bile ducts and portal veins. The parenchyma of liver at high dose of imidacloprid for 30 days showed cytoplasmic vacuolation and hyperchromasia in hepatocytes. There were focal areas of necrosis in the parenchyma surrounded by neutrophilic infiltration. Periportal reaction is also evident with infiltration of the neutrophils in the periportal region particularly around the portal veins.

A large number of xenobiotics have capability to generate free radicals in biological system raising question whether oxidative stress is major concern for tissue damage. Imidacloprid has found maximum concentration in liver and kidney in time dependant manner and metabolized mainly in liver and 75% was found in urine after 48 hours (Cordone and Dunkin, 2005). However, antioxidant enzymes like SOD and GPx may have effect on oxidant molecules on tissues and are active in defence against oxidative cell injury by means of their being free radical scavengers. Pesticides mediated toxicity involves excessive production of ROS leading to the alteration in the cellular antioxidant defence system and consequently affecting susceptibility to oxidative stress (Lopez et al., 2007). Lipids containing polyunsaturated fatty acids have abundant sites for ROS because they have double bonds between carbon atoms. In present study, imidacloprid significantly induced LPO and decreased other vital antioxidant in liver at high dose rate. High level of LPO in liver suggested the production of oxidative metabolites or free radicals during hepatic metabolism and this might be due to progressive nature of free radical chain reaction. Increased level of MDA in tissues supports the results of Giray et al., (2001) and Kalender et al., (2006). Increase in relative organ weight may occur as imidacloprid is rapidly absorbed via the gastrointestinal tract (Meister, 1994) and the liver is the main organ to metabolize this compound.

Glutathione (GSH) is the most abundant non-protein thiol in organisms and it plays a key role in intracellular protection against toxic compounds, such as...
reactive oxygen intermediates and other free radicals (Anderson and Luo, 1998). GSH plays a major role in antagonizing the oxidative action of the herbicides or insecticides (Parke and Piotrowski, 1996). It was found that imidacloprid significantly suppressed GSH concentration in the liver which may be evidence for depressed antioxidant capacity by imidacloprid. Antioxidant enzymes such as SOD and GSH-Px play an important role in the protection against deleterious effects of lipid peroxidation. SOD constitute first line of defence against deleterious effects of oxyradicals in cell by catalysing dismutation of superoxide radical and second line of defence constitute by GSH-px. In present study SOD level is significantly decreased in higher doses. Decrease in activity of SOD in liver might be due to consumption of this enzyme in $O_2^-$ to $H_2O_2$. Similar decreased in activity of SOD in animals was also reported with different pesticides namely Chlorpyrifos, Cypermethrin, Carbofuran, Dimethoate and Malathion which showed decreased SOD activity in rats (Khan et al., 2005; Rai and Sharma, 2007; Mansour and Mossa, 2009). The second barrier is provided by GSH-Px because of its lower Km for $H_2O_2$ and the third by catalase (Debanath and Mandal, 2000). GSH-Px is localized mainly in cytosol and mitochondria of liver, so this organ can be accepted as source of this enzyme. Therefore, decreased GSH-Px activity in liver might be due to oxidative inactivation of the enzyme protein because of the accumulation of insecticide in liver (El-Tawil and Abdel, 2001; Giray et al., 2001).

**Fig. 1:** Histopathological findings due to imidacloprid in liver (10x)

- **a** Normal hepatocytes of control group.
- **b** Mild cellular swelling in the hepatocytes and sinusoidal dilation.
- **c** Congestion in vein.
- **d** Focal areas of necrosis in the parenchyma surrounded by neutrophilic infiltration.
- **e, f** Infiltration of the neutrophils around bile ducts and portal vein.
In present study, decrease in both cytoplasmic and membrane bound proteins in liver in imidacloprid treated rats were found. Significant decrease in protein level might be due to catabolism of protein and/or malfunction of liver (Harper et al., 1977). It has been found that rapid loss in proteins of the brain during pesticide toxicity was reported by Richardson (1981). Swamy et al., (1992) have reported that the decrease in total proteins and soluble proteins indicate their metabolic utilization. This indicates that free radicals not only alter the lipid bilayer but also affects the activities of various membrane bound enzymes (Rhodes et al., 1984; Shakoori et al., 1992). Findings of histopathological studies were also in accordance with histopathological lesions observed in livers of male rats (Mohany et al., 2011), Japanese quail (Omiiana, 2004) and in layer chickens (Kammon et al., 2010).

Epidemiological studies suggested that exposure of pesticides may increase prevalence of respiratory diseases (Salameh et al., 2003), neurological dysfunctions (Paolini et al., 2004), cancers (Flower et al., 2004) and reproductive disorders (Kumar, 2004).

The results of present study indicated that imidacloprid had not induced oxidative stress at 19 mg/kg/day dose to Wistar albino rats when exposed for period of 10,20 and 30 days. However imidacloprid at 38 mg/kg/day had significantly induced oxidative stress to rats. This might be due to disturbance in cellular oxidative status as evidenced by increased level of LPO, decreased activities of SOD, GPx, GSH levels, cytoplasmic and membranic proteins and histopathological changes in liver tissues.

REFERENCES


