Sub-acute oxidant and histopathological effects of imidacloprid on kidney of adult female Wistar rats

Archana Lohiya*, Vinod Kumar and J.S. Punia

Department of Veterinary Pharmacology and Toxicology,
Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125 001, Haryana, India.

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ABSTRACT

Imidacloprid is a neonicotinoid insecticide and has been extensively used as a crop pest and pet flea control programme. In the present study, the effects of imidacloprid on kidney tissue were analyzed in adult female Wistar 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 animals administered daily with 2% gum acacia. Different parameters undertaken were kidney weight, levels of cytoplasmic and membrane proteins, oxidative stress parameters viz. activities of SOD, GPx and levels of GSH and MDA and histopathological changes. Imidacloprid at 38 mg/kg dose administered orally for 20 and 30 days significantly increased organ weight and levels of MDA. The activities of enzymes SOD and GPx and levels of GSH were decreased significantly at 38 mg/kg dose administered daily for 30 days. There was significant decrease observed in levels of cytoplasmic and membrane proteins at 38 mg/kg dose administered orally for 20 and 30 days. Histopathological changes in kidney at 38 mg/kg dose in 20 and 30 days group. Therefore, it is concluded that imidacloprid has generated oxidative stress in kidney at 38 mg/kg dose administered orally for 20 and 30 days in adult female Wistar rats.

Key words: Female rats, Histopathology, Imidaclorpid, Kidney, Oxidative stress.

INTRODUCTION

Imidacloprid was the first representative of neonicotinoid insecticides to be registered for use by USEPA. After introduction in the market in 1992, it ranked as one of the top selling pesticides in the world in 2001-2002. Imidacloprid is registered worldwide in more than 120 countries and in over 140 crops for agricultural use particularly in rice, cereal, maize, potatoes, vegetables, sugar beets, fruit, cotton, hops and turf. Imidacloprid which act selectively on nicotinic acetylcholine receptors (nAChRs), is used for insect pest management and flea control in cats and dogs (Abou Donia et al., 2008). The selective toxicity to insects over vertebrates of neonicotinoids has been shown to result from their selectivity to insect nAChRs (Matsuda et al., 2001; Tomizawa et al., 2000). However, even having the selective toxicity to insects, the excessive use of imidacloprid for a prolonged time period may lead to adverse and toxic effects. Inordinate, indiscriminate and injudicious use of these compounds can be fatal for human as well as animal health.

Since kidney is associated with elimination of toxicants from 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 Indofil chemical company Mumbai, India and all biochemicals used in experiment were procured from Analytical Rasayan and Himedia Laboratories Pvt. Ltd.

Experimental design: The study was conducted on sexually mature female Wistar rats, weighing 120–140 g obtained from Disease Free Small Animal House (DFSAH) of Lala Lajpat Rai University of Veterinary and Animal Sciences (LUVAS), Hisar. Approval from institutional animal ethical committee was obtained for use of animals. The animals were housed in groups of six 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. Rats were randomized divided into nine groups of six rats per group. 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. The doses i.e. 19 and 38 mg/kg/day were 1/20th and 1/10th of LD_{50}, (Median Lethal Dose) respectively (Tomlin, 2006; NPIC, 2010).

In control, one group was randomly chosen and sacrificed, using ether anesthesia (Booth Veterinary pharmacology and therapeutics, 6th ed) 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 ether anesthesia, on day 10, 20 and 30 of daily administration of imidacloprid at 19 and 38 mg/kg/day dose, respectively.

Tissue homogenate preparation: Kidney was quickly removed, trimmed of extraneous tissue and washed with ice cold physiological saline solution and then weighed. After that kidney 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 of 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 MEASUREMENTS

LPO: The formation of thiobarbituric acid-reactive substances (TBARs) in kidney was monitored as an index of lipid peroxidation according to a previously described colorimetric method (Ohkhawa et al., 1972).

Procedure: Briefly, 0.1 ml of 10% tissue homogenate prepared in 1.15% KCl solution then added 0.2 ml of 8.1% SDS solution. Then add 1.5 ml of 20% acetic acid solution and was adjusted to 3.5 pH and then 1.5 ml of 0.8% aqueous solution of TBA was added. Distilled water was added to make final volume to 4.0 ml. The reaction mixture was heated in water bath at 95°C for 60 min and then cooled. To this added 1.0 ml of distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1). It was shaken vigorously and centrifuged at 4000 rpm for 10 min. The organic layer was separated and the absorbance of organic layer was taken at 532 nm. Different concentration of TMP was taken and standard curve was prepared. Results are expressed as nanomoles of MDA per gram of wet tissue.

GSH: GSH level in tissue homogenate was measured by method of Beutler, (1963). This method depends on the reaction of reduced glutathione with 5, 5-dithiobis, 2- (nitrobenzoic acid) that can be measured spectrophotometrically. The yellow colour developed was measured at 412 nm against a blank reagent. The results are expressed as μmol/mg protein.

Procedure
• m-phosphoric acid precipitation solution: Dissolve 1.67 g glacial metaphosphoric acid, 0.2 g EDTA and 30 g sodium chloride in 100 ml of distilled water.
• Phosphate solution: 0.3 M disodium hydrogen phosphate (Na,HPO_{4}).
• DTNB Reagent: Dissolve 40 mg 5, 5-dithiobis 2- (nitrobenzoic acid) in 100 ml of 1% aqueous trisodium citrate solution.

0.2 ml of 10% tissue homogenate (prepared in phosphate buffer, containing 0.02 M EDTA) was added to 1.8 ml of distilled water. 3.0 ml of precipitating solution was mixed. It was mixed and allowed to stand for approximately 5 min and then centrifuged. Two ml of filtrate was added to 8.0 ml of the phosphate solution. A blank was prepared with 8.0 ml of the phosphate solution, 2.0 ml of the diluted precipitating solution (3 parts precipitating solution + 2 parts distilled water). 1.0 ml of the DTNB reagent was added to sample and blank tubes. Standard solutions of different concentration of GSH were prepared in phosphate buffer containing 0.02 M EDTA solution. The yellow colour developed was measured at 412 nm against a blank reagent. The results are expressed as μmol/mg protein.

GPs: GSH-Px activity was measured by method of Hafeman et al., (1974) which was modification of Mills procedure. The GSH-Px catalyzes the reaction between H_{2}O_{2} and reduced glutathione to form oxidized glutathione and water. The rate of oxidation of GSH by H_{2}O_{2} was used as measure of GPx activity. Results are expressed as U/mg protein.

Procedure
• 2.0 mM reduced glutathione solution.
• 0.4 M sodium phosphate buffer solution containing 4x10^{-4} M EDTA.
• 0.01 M sodium azide (NaN_{3}) solution.
• 1.2 mM H_{2}O_{2} solution.
• 0.4 M disodium hydrogen phosphate (Na,HPO_{4}) solution.
• m-phosphoric acid precipitation solution: Dissolve 1.67 g glacial metaphosphoric acid, 0.2 g EDTA and 30 g sodium chloride in 100 ml of distilled water.
• DTNB reagent: Dissolve 40 mg 5, 5-dithiobis 2- (nitrobenzoic acid) in 100 ml of an aqueous 1% trisodium citrate solution.

Tissue homogenate was prepared in 0.15 M KCl solution. 0.2 ml of reduced glutathione solution, 0.2 ml of
buffer and 0.1 ml of sodium azide solution was taken in sample, control and blank tubes. 0.1 ml of tissue homogenate and 0.2 ml of distilled water was added to sample and control tubes. To blank tube only 0.3 ml of distilled water was added. After 5 min of preincubation at room temp (25 °C) 0.2 ml of prewarmed H₂O₂ (at 37 °C) was added to sample and blank except control tube to which 0.2 ml of distilled water was added. After 3 min interval, 4.0 ml of metaphosphoric acid precipitation solution was added to all the three tubes and centrifuged. From the above centrifuged tubes 2.0 ml of filtrate was pippeted out and was added with 2.0 ml of 0.4 M disodium hydrogen phosphate solution and 0.1 ml of DTNB reagent and mixed thoroughly. Absorbance was read at 420 nm.

**SOD:** Superoxide dismutase (SOD) activity was estimated as per the method described by Madesh and Balsubramaniam (1998). It involves the generation of superoxide by pyrogallol autoxidation and inhibition of superoxide dependent reduction of the tetrazolium dye MTT [3-(4-5 dimethyl thiozol 2-yl) 2, 5-diphenyl tetrazolium bromide] to its formazen, which is measured at 570 nm. The reaction was terminated by the addition of dimethyl sulfoxide (DMSO), which helps to solubilize the formazen formed. The colour evolved was stable for many hours and was expressed as SOD Units (one unit of SOD is the amount of (µg) protein required to inhibit the MTT reduction by 50%). Results are expressed as U/mg protein.

**Procedure**

- Pyrogallol (100 µM) solution: 6.3 mg of pyrogallol was dissolved in 5 ml of distilled water. One ml of this solution was diluted to 100 ml with distilled water.
- MTT (1.25 mM) solution: 2.58 mg MTT was dissolved in 5 ml of distilled water.
- Phosphate buffer saline (PBS): PBS was prepared by dissolving NaCl (8 gm), KCl (0.2 gm), KH₂PO₄ (0.2 gm) and Na₂HPO₄ (0.94 gm) in about 800 ml distilled water. pH was adjusted to 7.4 and volume was made up to 1 litre with distilled water.

10% Tissue homogenate was prepared in phosphate buffered saline and centrifuged. The supernatant was used for estimation of SOD enzyme activity. 0.65 ml of PBS and 30 µl of MTT solution were taken in sample, control and blank tubes. Then 10 µl of lysate or tissue homogenate was added in sample test tube. Then 75 µl of Pyrogallol solution was added to sample, control and blank tubes. The tubes were incubated for 5 min at room temperature. Then after 5 min, 0.75 ml of DMSO was added in all the three tubes. Finally 10 µl of tissue homogenate or lysate was added to control tube. These solutions were thoroughly mixed and read absorbance at 570 nm.

**Estimation of proteins:** The levels of cytoplasmic and membranic proteins estimated in kidney using Autopak kits in clinical analyser machine. 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 kidney was done according to Luna, (1968). A portion of the specimen was fixed in 10% formosaline solution for at least a week period for histopathological studies. Then samples were subjected to overnight washing, the specimens were then dehydrated in ascending grade of alcohol, cleared in benzene and then embedded in paraffin wax to prepare paraffin blocks. Then 5µm thick sections were cut with microtome and subjected to further processing and finally stained with hematoxylin and eosin (H/E stain). 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 kidney weight:** There was significant(p<0.05) increase in the values of relative kidney weight of female rats treated with imidacloprid at 38 mg/kg dose orally daily for 20 and 30 days, respectively. However, no significant difference was observed in rats treated with imidacloprid 19 mg/kg orally for 10, 20 and 30 days and rats treated with 38 mg/kg for 10 days (Table 1).

**Effect on LPO:** There was significant(p<0.05) increase in the values of MDA levels in tissue homogenate of kidney of female rats treated with imidacloprid at 38 mg/kg dose orally daily for 20 and 30 days, respectively. However, no significant difference was observed in rats treated with imidacloprid 19 mg/kg orally for 10, 20 and 30 days and rats treated with 38 mg/kg for 10 days (Table 2).

**Effect on GSH:** A significant (p<0.05) decrease in the value of reduced glutathione level in tissue homogenate of kidney of female rats treated with imidacloprid at 38 mg/kg dose orally daily for 30 days was observed. However, no significant difference was observed in rats treated with imidacloprid 19 mg/kg orally for 10, 20 and 30 days and rats treated with 38 mg/kg for 10 and 20 days, respectively (Table 3).

**Effect on GPx:** A significant (p<0.05) decrease in the activity of glutathione peroxidase enzyme in tissue homogenate of kidney of female rats treated with imidacloprid at 38 mg/kg dose orally daily for 30 days was observed. However, no significant difference was observed in rats treated with
Table 1: Effect of toxicity of imidacloprid on relative weight of kidney (g/100g) of adult Female Wistar rats.

<table>
<thead>
<tr>
<th>Treatment (mg/kg, p.o.)</th>
<th>Kidney</th>
<th>Duration of treatment (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Control (200)</td>
<td>0.25±0.004</td>
<td>0.26±0.004</td>
</tr>
<tr>
<td>IMI (19)</td>
<td>0.24±0.004</td>
<td>0.25±0.007</td>
</tr>
<tr>
<td>IMI (38)</td>
<td>0.25±0.007</td>
<td>0.29±0.006*</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) levels in kidney of adult Female Wistar rats.

<table>
<thead>
<tr>
<th>Treatment (mg/kg, p.o.)</th>
<th>Kidney</th>
<th>Duration of treatment (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
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<tr>
<td>Control (200)</td>
<td>178.21±2.45</td>
<td>178.26±1.86</td>
</tr>
<tr>
<td>IMI (19)</td>
<td>178.66±1.17</td>
<td>180.16±2.43</td>
</tr>
<tr>
<td>IMI (38)</td>
<td>180.06±2.38</td>
<td>187.26±2.22*</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 GSH levels (µmol/mg protein) in kidney of adult Female Wistar rats.

<table>
<thead>
<tr>
<th>Treatment (mg/kg, p.o.)</th>
<th>Kidney</th>
<th>Duration of treatment (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Control (200)</td>
<td>0.13 ±0.009</td>
<td>0.12 ±0.020</td>
</tr>
<tr>
<td>IMI (19)</td>
<td>0.12 ±0.016</td>
<td>0.11 ±0.012</td>
</tr>
<tr>
<td>IMI (38)</td>
<td>0.11 ±0.008</td>
<td>0.09 ±0.012</td>
</tr>
</tbody>
</table>

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

Table 4: Effect of toxicity of imidacloprid on activity of GPx (U/mg protein) enzyme in kidney of adult Female Wistar rats.

<table>
<thead>
<tr>
<th>Treatment (mg/kg, p.o.)</th>
<th>Kidney</th>
<th>Duration of treatment (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Control (200)</td>
<td>10.5±0.20</td>
<td>10.1±0.25</td>
</tr>
<tr>
<td>IMI (19)</td>
<td>10.9±0.24</td>
<td>9.8±0.18</td>
</tr>
<tr>
<td>IMI (38)</td>
<td>9.9±0.29</td>
<td>9.6±0.17</td>
</tr>
</tbody>
</table>

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

Table 5: Effect of toxicity of imidacloprid on activity of SOD enzyme (U/mg protein) in kidney of adult Female Wistar rats.

<table>
<thead>
<tr>
<th>Treatment (mg/kg, p.o.)</th>
<th>Kidney</th>
<th>Duration of treatment (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>Control (200)</td>
<td>8.16±0.94</td>
<td>7.32±0.56</td>
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<tr>
<td>IMI (19)</td>
<td>7.62±0.56</td>
<td>6.5±0.69</td>
</tr>
<tr>
<td>IMI (38)</td>
<td>6.33±0.54</td>
<td>5.2±0.94</td>
</tr>
</tbody>
</table>

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

imidacloprid 19 mg/kg orally for 10, 20 and 30 days and rats treated with 38 mg/kg for 10 and 20 days, respectively (Table 4).

Effect on SOD: A significant (p<0.05) decrease in the activity of SOD enzyme in tissue homogenate of kidney of female rats treated with imidacloprid at 38 mg/kg dose orally daily for 30 days was observed. However, no significant difference was observed in rats treated with imidacloprid 19 mg/kg orally for 10, 20 and 30 days and rats treated with 38 mg/kg for 10 and 20 days, respectively (Table 5).

Effect on Cytoplasmic proteins: A significant (p<0.05) decrease in the value cytoplasmic protein levels in tissue homogenates of kidney of female rats treated with imidacloprid at 38 mg/kg dose orally daily 20 and 30 days was observed (Table 6).

Effect on Membrane proteins: A significant (p<0.05) decrease in the value of membrane protein levels in tissue homogenates of kidney of female rats treated with imidacloprid at 38 mg/kg dose orally daily for 20 and 30 days was observed (Table 7).

Histopathological Findings in kidney: Histopathological findings due to imidacloprid in kidney are represented in Fig. 1. No pathological changes were observed in kidney of female rats exposed to IMI (19) at for 10, 20 and 30 days of exposure.
Table 6: Effect of toxicity of imidacloprid on cytoplasmic protein levels (mg/g tissue) in kidney of adult Female Wistar rats.

<table>
<thead>
<tr>
<th>Treatment (mg/kg, p.o.)</th>
<th>Kidney</th>
<th>Duration of treatment (days)</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (200)</td>
<td></td>
<td></td>
<td>70.21±1.86</td>
<td>68.19±2.43</td>
<td>66.03±1.39</td>
</tr>
<tr>
<td>IMI (19)</td>
<td></td>
<td></td>
<td>68.19±1.86</td>
<td>65.03±1.29</td>
<td>61.72±1.87</td>
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<tr>
<td>IMI (38)</td>
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<td></td>
<td>66.07±1.50</td>
<td>60.15±1.96*</td>
<td>55.13±2.43*</td>
</tr>
</tbody>
</table>

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

Table 7: Effect of toxicity of imidacloprid on membrane protein levels (mg/g tissue) in kidney of adult Female Wistar rats.

<table>
<thead>
<tr>
<th>Treatment (mg/kg, p.o.)</th>
<th>Kidney</th>
<th>Duration of treatment (days)</th>
<th>10</th>
<th>20</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (200)</td>
<td></td>
<td></td>
<td>25.15±2.01</td>
<td>24.14±2.08</td>
<td>23.22±1.51</td>
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<tr>
<td>IMI (19)</td>
<td></td>
<td></td>
<td>23.50±1.10</td>
<td>22.11±1.96</td>
<td>20.21±1.97</td>
</tr>
<tr>
<td>IMI (38)</td>
<td></td>
<td></td>
<td>21.09±2.38</td>
<td>18.12±1.29*</td>
<td>15.15±2.22*</td>
</tr>
</tbody>
</table>

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

Fig. 1: Histopathological findings due to imidacloprid in kidney (10x)
   a: Normal microscopic picture of kidney of control group.
   b: Congestion in blood vessels.
   c: Focal necrosis of tubules was present with infiltration of neutrophils and lymphocytes in the parenchyma.
   d: Degenerative changes like cloudy swelling in the tubular epithelium, contracted glomeruli.
Repeated exposure of IMI (38) for 20 days produces degenerative changes like cloudy swelling in the tubular epithelium, contracted glomeruli. At many places, focal necrosis of tubules was present with infiltration of neutrophils and lymphocytes in the parenchyma. Repeated exposure of IMI (38) for 30 days also produces degenerative changes like vacuolation in the tubular epithelium along with sloughing of epithelial cells in the lumen. Focal necrosis of tubules was also present with infiltration of neutrophils and lymphocytes in the parenchyma.

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 defense against oxidative cell injury by means of their being free radical scavengers. Pesticides mediated toxicity involves excessive production of ROS (Reactive oxygen species) leading to the alteration in the cellular antioxidant defense system and consequently affecting susceptibility to oxidative stress (Lopez et al., 2007). Pesticide induced oxidative radicals leads to DNA damage, protein degradation, LPO (lipid peroxidation) and finally culminating into damage to various vital tissues like liver, kidney and brain. 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 antioxidants in kidney at high dose rate. High level of MDA in kidney of rats administered with imidacloprid suggested the production of oxidative metabolites or free radicals. This may be due to progressive nature of free radical chain reaction.

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 et al., 1998). GSH plays a major role in antagonizing the oxidative action of the herbicides or insecticides (Parke and Dunkin, 1996). We found that imidacloprid significantly suppressed GSH concentration in the kidney which may be evidence for depressed antioxidant capacity by imidacloprid. Antioxidant enzymes such as SOD and GSH-Px which act as preventative antioxidants 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. In present study SOD level is significantly decreased in higher doses. Decrease in activity of SOD in kidney of imidacloprid intoxicated rats may be due to consumption of this enzyme in O2 to H2O2. Similar decreased in activity of SOD in animals was also reported with different pesticides namely chlorpyrifos, Cypermethrin, Carbofuran, Dimethoate and malathion which shows decrease SOD activity in rats (Khan et al., 2005; Rai and Sharma, 2007; Mansour and Mossa, 2009). The toxic end product of SOD, has to be removed by GSH-Px. The second barrier is provided by GSH-Px because of its lower Km for H2O2 and the third by catalase (Debanath and Mandal, 2000). Therefore, decreased GSH-Px activity in kidney may be due to oxidative inactivation of the enzyme protein because of the accumulation of insecticide in this tissue (El-Tawil and Abdel, 2001; Giray et al., 2001).

The decrease activity of SOD, GPx and GSH content together with increase LPO may be attributed to induce free radicals in high dose imidacloprid treated rats. In present study, decrease in both cytoplasmic and membrane bound proteins in kidney in imidacloprid treated rats were found. Significant decrease in protein level may indicate the induced degenerative changes in kidney of rats or general disturbance in the protein anabolism. (Harper et al., 1977). It has been found that rapid loss in proteins of the brain during pesticide toxicity was reported (Richardson, 1981). The histopathological changes seen in kidney of rats administered with imidacloprid 38 mg/kg for 20 and 30 days also suggests that imidacloprid induce degenerative changes in kidney of adult female Wistar rats.

The toxicity of many xenobiotics is associated with the production of free radicals which are not only toxic to themselves, but may also implicated in the pathophysiology of various diseases (Abdollahi et al., 2004). Epidemiological studies suggest 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 has not induced oxidative stress at 19 mg/kg/day dose to female rats when exposed for period for 10, 20 and 30 days. However imidacloprid at 38 mg/kg/day dose has significantly induced oxidative stress in female rats. This may 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 kidney tissues.

REFERENCES


