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Effect of imidacloprid on hepatotoxicity and nephrotoxicity in male albino mice

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ABSTRACT

Imidacloprid (IC) is a systemic insecticide related to the tobacco toxin nicotine. IC is a toxic substance frequently used into combat insects, rodents and plants pests and other creatures that can pose problems for agriculture. We, therefore, planned this study to assess risk factors, biochemical and histological alterations associated with hepatotoxicity and nephrotoxicity. Forty-eight adult male albino mice were divided into four groups of 12 animals each. All the animals were given standard synthetic pellet diet. One group served as control, and the other three were served as experimental groups. Decrease in the body weight of the high dose group was observed at 15 mg/kg/day, and no mortality occurred during the treatment period. High dose of imidacloprid caused a significant elevation of serum clinical chemistry parameters, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate kinase (SGPT), alkaline phosphatase (ALP) and total bilirubin (TBIL). Histology of liver and kidney indicates hepatotoxicity and nephrotoxicity at a high dose of imidacloprid. Based on the morphological, biochemical and histopathological analysis, it is evident that imidacloprid induced toxicological effects at 15 mg/kg/day to mice. The results of the present study demonstrate that IC had significant effects on body weight, liver functions and kidney ($p < 0.05$) at a dose of 15 mg/kg body weight. IC treatment 5 and 10 mg/kg/day may be considered as no observed adverse effect level (NOAEL) for mice. It was concluded that IC can cause hepatotoxicity and nephrotoxicity at a dose much lower than the LD_{50} (131 mg/kg body weight) in mice.

1. Introduction

Fruits and vegetables are an essential part of a nutritious and healthy diet; however, the health benefits are compromised by consistent contamination with pesticide residues. In our previous work, pesticide was detected in a range of fresh vegetables [1]. These highly stable compounds can last for years or decades before breaking down. They

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circulate globally and persistent pesticides released in one part of the world can, through a repeated process of evaporation and deposit, be transported through the atmosphere to the areas far away from the source [2]. Human health risks vary with the type of the pesticides and also with the extent of vulnerability. Immediate human health hazards from pesticides include mild headaches, flu, skin rashes, blurred vision and other neurological disorders, and rarely, paralysis, blindness, and even death. Long run health impacts include cancer, infertility, miscarriage, male sterility, birth defects, and effects on the nervous system [3]. Pesticides can also interfere with drug-metabolizing enzymes, especially Cytochrome P450 leading to drug interactions [4].

The liver cytochrome P450 (CYPs) is the major enzymes involved in drug metabolism, accounting for ~75% of the total metabolism [5] and activation or detoxification of neonicotinoids [6]. Studies of the metabolites of neonicotinoids have shown that they can be bioactive and act as nAChR agonists or cause secondary toxicity in mammals [7]. Nicotinoids can be formed as metabolites of neonicotinoids with greater selectivity for vertebrate nAChRs than to insect nAChRs [6,7].

Nephrotoxicity of pesticides has been reported in mice [8] and in rats [9]. Imidacloprid, 1[(6-chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine, a chloronicotyl is an extensively used insecticide for crop protection in the world wide for the last decade due to its low soil persistence and high insecticidal activity at low application rate [10]. Imidacloprid, nitenpyram, acetamiprid, thiacloprid, thiamethoxam, and others act as agonists at the insect nicotinic acetylcholine receptor (nAChR) [6]. It is the fastest growing in sales because of its high selectivity for insect nicotinic acetylcholine receptors [11]. IC is most widely used neonicotinoids insecticide in agriculture can exaggerate the toxic properties and adverse effects of insecticide and can be fatal for human as well as animal health. Buckingham et al. showed that imidacloprid affects both AChRs sensitive to -BTX and -BTX-insensitive nicotinic acetylcholine receptors (nAChRs) can act on pharmacologically diverse nAChR subtypes [12]. Oral LD50 of imidacloprid is 131 mg/kg in mice. It is rapidly absorbed from the gastrointestinal tract and eliminated via urine and feces [13].

In the present study, we examined possible effects of imidacloprid on hepatotoxicity and nephrotoxicity by using biochemical and histological techniques in mice.

2. Materials and methods

Forty-eight adult male albino mice weighing 25 to 30 g were obtained for this study. The mice were obtained from the Experiment Animal Center of the Fourth Military Medical University (Xi'an, Shaanxi, China) and housed six per cage in our lab for 15 days before the experiment. This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals. All experimental procedures followed the principle of laboratory animal care and were carried out according to a protocol approved by the local animal ethics committee. All surgery was performed under sodium pentobarbital anesthesia, and all efforts were made to minimize suffering.

2.1. Experimental design

The adult male mice were randomly divided into a total of four groups 12 in each group. One group served as control and the other three groups were served as experimental groups, given 5, 10 and 15 mg/kg body weight imidacloprid by an oral gavage method for 15 days. The animals were maintained under conditions of controlled temperature (22 ± 3) and humidity (30–70%) with 12 h light and dark cycle. The animals were given standard synthetic pellet diet.

2.2. Chemicals

Commercial Imidacloprid 20% EC formulation, with the name Confidor was obtained from Bayer. Kits for SGOT, SGPT, ALP and TBIL were purchased from Human, Germany.

2.3. Sign of toxicity and mortality

Signs of toxicity such as salivation, lacrimation, diarrhea, tremor, convulsion, paralysis and death were observed once daily throughout the period of exposure. After 15 days of dosing mice, blood was collected in non-oxalate tubes for the separation of serum.

2.4. Urine examination

Urine of the individual animals was collected and arranged group wise, initially (day 0) before exposure, at day 5, 10 and finally at 15 days of treatment for the qualitative analysis of pH, specific gravity, presence of blood, glucose, bilirubin and protein by the help of the automatic urine analyzer (Urometer 600, Japan). Urine was collected by using metabolic cages in animal house.

2.5. Biochemical analysis

Blood samples were collected from all mice of the control and experimental group. The blood samples were centrifuged at $1500 \times g$ for 10 min after standing for 2 h. The serum was separated and immediately frozen to $-80\,^{\circ}C$ until analysis. Serum GOT, GPT, ALP and TBIL (biochemical parameters for LFTs) were measured through fully automated biochemical analyzer using standard kits (Human, Germany).

2.5.1. Serum creatinine and blood urea nitrogen (BUN) analysis

For creatinine, and blood urea nitrogen (BUN) plasma obtained in the above step was used. Creatinine, and blood urea nitrogen (BUN) concentrations were determined in plasma. Plasma creatinine was measured by used high-performance liquid chromatography (HPLC) methods in mice [14]. Samples were additionally spiked with 10 μl of a creatinine standard stock solution in 0.2 N HCl (Sigma, Munich, Germany) or 10 μl 0.2 N HCl to controls. Renal function is assessed by serum creatinine (SCR)

and blood urea nitrogen (BUN) reflects the glomerular filtration rate (GFR) poorly in mild or moderate renal impairment. BUN was measured according to Roch-Camel with an Automated Clinical Chemistry Analyser (ADVIA Chemistry System 2400, Siemens Healthcare Diagnostics, Erlangen, Germany) [15]. In brief, urea was hydrolyzed to ammonia and carbon dioxide by urease. In the following indicator reaction, glutamate-dehydrogenase catalyzed the synthesis of glutamate and NAD from ammonia and alpha-ketoglutarate. The oxidation of NAD from NADH was measured by a decrease in the absorbance at 340 nm.

2.6. Histopathological examination

Representative samples from the liver and kidneys were collected at 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 (H) and eosin (E) for microscopic examination. Histology of liver and kidney were achieved by using H and E staining.

2.7. Statistical analysis

Statistically significant differences were determined using one-way ANOVA with Newman–Keuls in Prism statistical software (GraphPad Software, San Diego, CA, USA). A $p < 0.05$ was considered significant in all cases. Data were reported as mean ± SD.

3. Results

3.1. Body weight

To search for the IC lowest observable effect level (LOEL) expressed in mice that have been previously shown to be a different value in a variety of experiment [11,16,17]. We assessed that repeated oral administration of imidacloprid at 5 and 10 mg/kg/day did not produce any signs of toxicity and mortality during 15 days exposure. However, there was a significant decrease in body weight of animals at the highest dose (15 mg/kg/day) together with significant toxicity symptoms as shown in Fig. 1 (Supplementary Table 1). In addition, we found (15 mg/kg/day) is the lowest observed effect level in mice.

3.2. Weight of liver and kidney

Acute exposure to various pesticides is associated with the structural damage to organs and tissues along with pathological and inflammatory changes resulting into altered morphology of affected organs. The values of weight measurements of various vital organs are presented in (Supplementary Table 2). There was a significant increased in weight of liver and kidney at the highest dose 15 mg/kg/day as shown in Fig. 2. The relative weight of the liver and kidney was significantly increased ($p < 0.05$) at 15 mg/kg/day doses (Table 1).

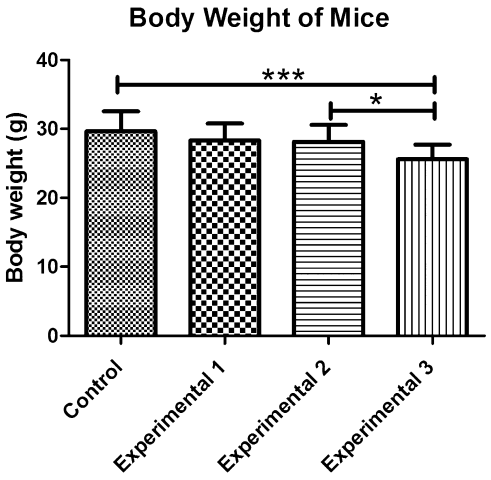

Fig. 1. Effect of imidacloprid treatment on body weight (g) of mice. Control animal fed on mice diet and the other three groups were served as experimental groups 1, 2 and 3, given 5 mg/kg, 10 mg/kg and 15 mg/kg body weight imidacloprid by an oral gavage method for 15 days, respectively. Weight associated changes with high dose represent that it has a toxic effect at this dose level in 15 days. Each value represents the mean ± SD for 12 mice with p value ($p < 0.05$).

3.3. Urine examination

As we found decreased in body weight and increased in the weight of liver and kidney (Supplementary Tables 1 and 2). We examined the counteracting effect of these organs on urine. Serum creatinine and blood urea nitrogen (BUN) determine the glomerular filtration rate (GFR) improperly in acute renal failure. Statistical significant changes in serum creatinine and BUN show renal damage in high dose exposure mice. There was no significant change in qualitative analysis of urine parameters like, protein, pH, specific gravity, blood, bilirubin and glucose in urine of mice obtained before exposure (initial '0′ day) and at 15 days of exposure of the IC (Table 2).

3.4. Biochemical analysis

To investigate the functional activity of liver enzymes we performed LFTs to check whether the IC disturbed the normal activity of the liver. We confirmed that a significant increase ($p < 0.05$) was noted in serum SGOT, SGPT, ALP and TBIL in animals exposed to 15 mg/kg body weight imidacloprid, it caused a significant rise in the enzyme levels and total bilirubin ($p < 0.05$) as shown in Figs. 3(a and b) and 4(a and b), respectively. However, there was no significant change in LFTs when mice exposed to the doses of 5 and 10 mg/kg body weight ($p > 0.05$). The results of serum biochemical parameters of mice orally administered imidacloprid for 15 days are shown in Table 3.

3.5. Histopathology

To gain further insight into the tissue, we performed histology of the primary exposed area of the mice including liver and kidney. To check the toxicity caused by IC we stained the tissue section by using H and E staining. The

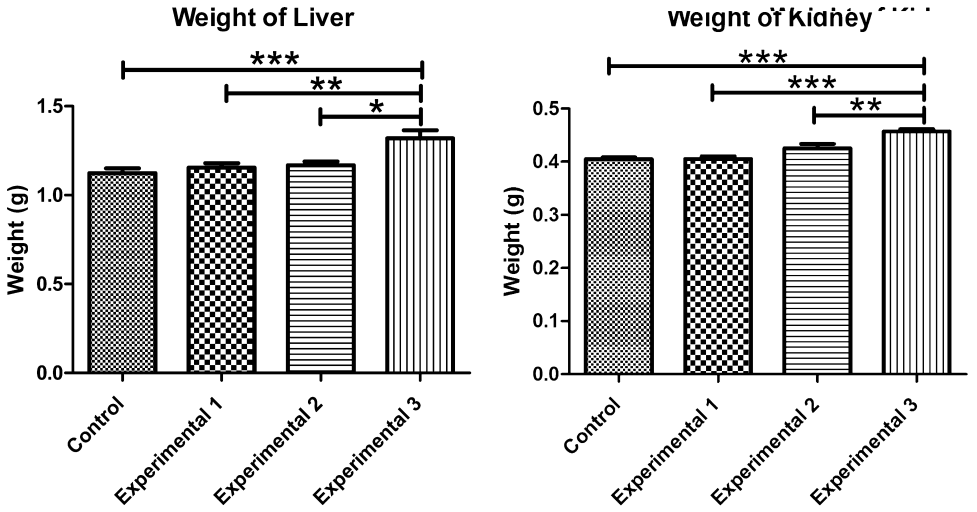

Fig. 2. Effect of imidacloprid treatment on Liver and kidney weight of mice. Control animal fed on mice diet and the other three groups were served as experimental groups 1, 2 and 3, given 5 mg/kg, 10 mg/kg and 15 mg/kg body weight imidacloprid by an oral gavage method for 15 days, respectively. Increase in the organ weight indicates toxicity of IC. Weight associated changes with high dose represent that it has a toxic effect at this dose level in 15 days. Each value represents the mean ± SD for 12 mice with p value ($p < 0.05$).

Table 1
Relative weight of liver and kidney.

Body organ	Relative weight of liver and kidney (g)			
	Control (Day 0) 0 (mg/kg/day)	Experimental 1 (Day 5) 5 (mg/kg/day)	Experimental 2 (Day 10) 10 (mg/kg/day)	Experimental 3 (Day 15) 15 (mg/kg/day)
Liver	4.09 ± 0.024	4.25 ± 0.052	4.49 ± 0.070	4.83 ± 0.067
Kidney	1.47 ± 0.009	1.53 ± 0.041	1.57 ± 0.025	1.61 ± 0.032

The relative organ weight is expressed as (organ weight (g)/body weight (g) × 100) of mice. Each value represents the mean ± SD for 12 mice with p value ($p < 0.05$).

Table 2
Plasma and urine analysis data of mice orally administered imidacloprid for 15 days.

Parameters	Control (Day 0) 0 (mg/kg/day)	Experimental 1 (Day 5) 5 (mg/kg/day)	Experimental 2 (Day 10) 10 (mg/kg/day)	Experimental 3 (Day 15) 15 (mg/kg/day)
Plasma creatinine (U/l)	0.28 ± 0.017	0.28 ± 0.018	0.29 ± 0.015	0.35 ± 0.018***
Blood urea nitrogen (BUN) (mg/dl)	23.65 ± 1.026	24.38 ± 0.841	25.74 ± 1.102	29.44 ± 1.077***
Protein (mg/dl)	6.91 ± 1.33	7.85 ± 0.95	9.89 ± 1.18*	12.89 ± 1.51***
pH	5.49	5.50	6.0	5.58
Specific gravity	1.03	1.01	1.02	1.03
Glucose (mg/dl)	Negative (−ve)	Negative (−ve)	Negative (−ve)	Negative (−ve)
Blood (RBC/μl)	Negative (−ve)	Negative (−ve)	Negative (−ve)	Negative (−ve)
Bilirubin (mg/dl)	Negative (−ve)	Negative (−ve)	Negative (−ve)	Negative (−ve)

Values represent group wise qualitative urine analysis, not individual animal data. Each value represents the mean ± SD for 12 mice with p value ($p < 0.05$).
*** Highly significant difference from control ($p < 0.01$).

Table 3
Effect of different doses of imidacloprid on biochemical parameters of liver functions in mice. Administration of pesticides, imidacloprid administered orally with different concentration indicates clinical signs of intoxication.

Parameter	Control (Day 0) 0 (mg/kg/day)	Experimental 1 (Day 5) 5 (mg/kg/day)	Experimental 2 (Day 10) 10/mg/kg/day	Experimental 3 (Day 15) 15 (mg/kg/day)
SGOT (U/l)	45.53 ± 2.70	42.00 ± 2.47***	43.52 ± 2.42***	55.38 ± 3.50***
SGPT (U/l)	35.20 ± 2.55	34.65 ± 2.18**	32.30 ± 2.22**	40.63 ± 3.31***
ALP (U/l)	176.5 ± 2.58	178.3 ± 2.16*	179.7 ± 2.16**	185.5 ± 3.50***
TBIL (mg/dl)	0.30 ± 0.011	0.29 ± 0.009***	0.31 ± 0.012***	0.36 ± 0.011***

Each value represents the mean ± SD for 12 mice with p value ($p < 0.05$).
* Significant difference ($P < 0.05$).
** Very significant.
*** Highly significant difference from control ($p < 0.01$).

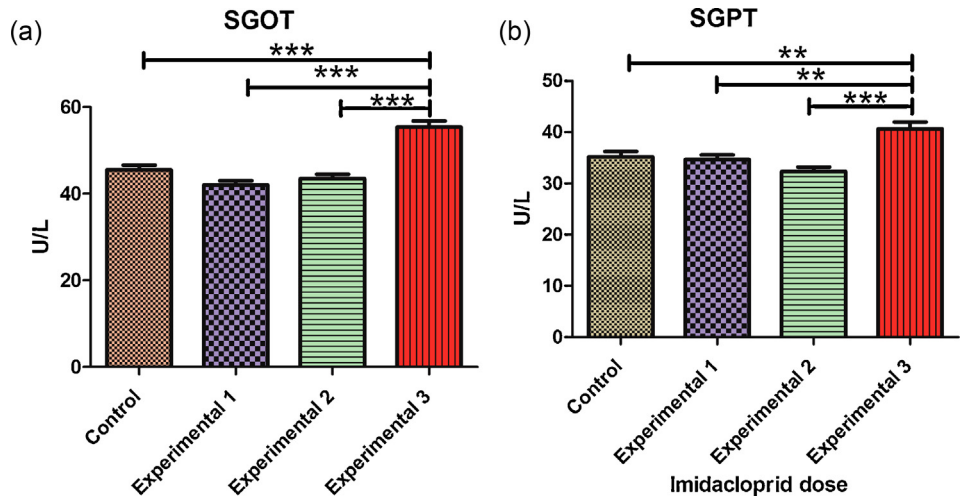

Fig. 3. Effect of different doses of imidacloprid on SGOT and SGPT levels in different groups. Control animal fed on mice diet and the other three groups were served as experimental groups 1, 2 and 3, given 5 mg/kg, 10 mg/kg and 15 mg/kg body weight imidacloprid by an oral gavage method for 15 days, respectively. (a) represents a high expression of SGOT levels in the liver while (b) represents the highest expression of SGPT levels in the liver. High value indicates the significant expression. Each value represents the mean ± SD for 12 mice with p value ($p < 0.05$).

liver and kidney tissues were placed in 10% buffered formalin. Thereafter, paraffin-embedded sections of these tissues were cut (5–6 μm thickness) and stained with H and E. The hepatocytes of the liver showed mild focal necrosis with swollen cellular nuclei, hypertrophied blood vessels and cytoplasmic lesions as shown in experimental mice (Fig. 5C and D) at repeated exposure of high dose of imidacloprid (15 mg/kg/d) for 15 days as compared with controls (Fig. 5A and B).

There was degeneration of the tubules, glomeruli and observed hemolysis in the kidney of the experimental mice as indicated in (Fig. 6C and D) at higher dose of imidacloprid (15 mg/kg/d) for 15 days as compared with controls (Fig. 6A and B). However, no pathological changes were observed in the liver and kidney of mice exposed to imidacloprid at 5 and 10 mg/kg/day doses.

Weight associated changes with high dose represents that it has a toxic effect at 15 mg/kg/day for 15 days.

4. Discussion

IC is most widely used neonicotinoids insecticide in agriculture for use on over 140 crops in more than 120 countries [18]. Such large-scale use in agriculture can exaggerate the toxic properties and adverse effects of insecticide and can be fatal for human as well as animal health. A quantitative analytical study disclosed that even a low level exposure to pesticide residues on fruits

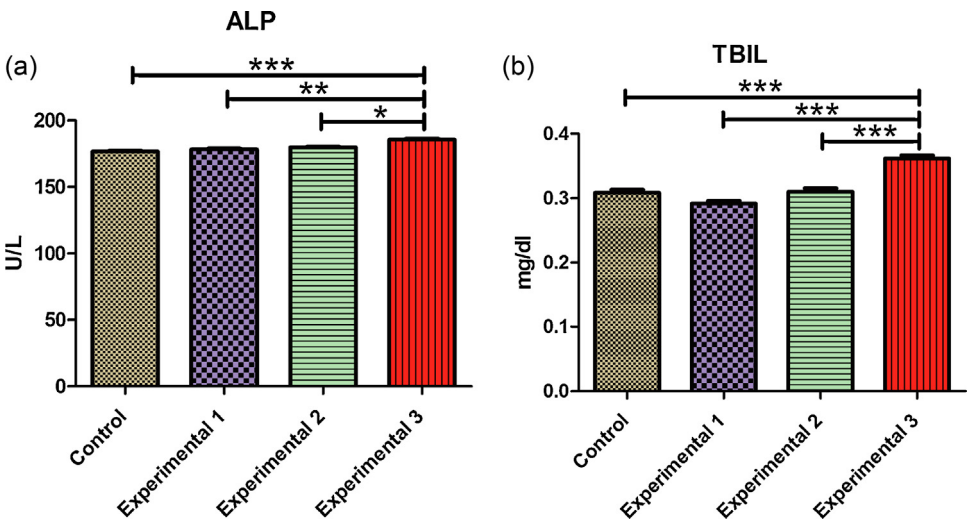

Fig. 4. Effect of different doses of imidacloprid on ALP and TBIL levels in different groups. Control animal fed on mice diet and the other three groups were served as experimental groups 1, 2 and 3, given 5 mg/kg, 10 mg/kg and 15 mg/kg body weight imidacloprid by an oral gavage method for 15 days, respectively. (a) represents high expression of ALP levels in the liver while (b) represents high expression of TBIL levels. High value indicates the significant expression. Each value represents the mean ± SD for 12 mice with p value ($p < 0.05$).

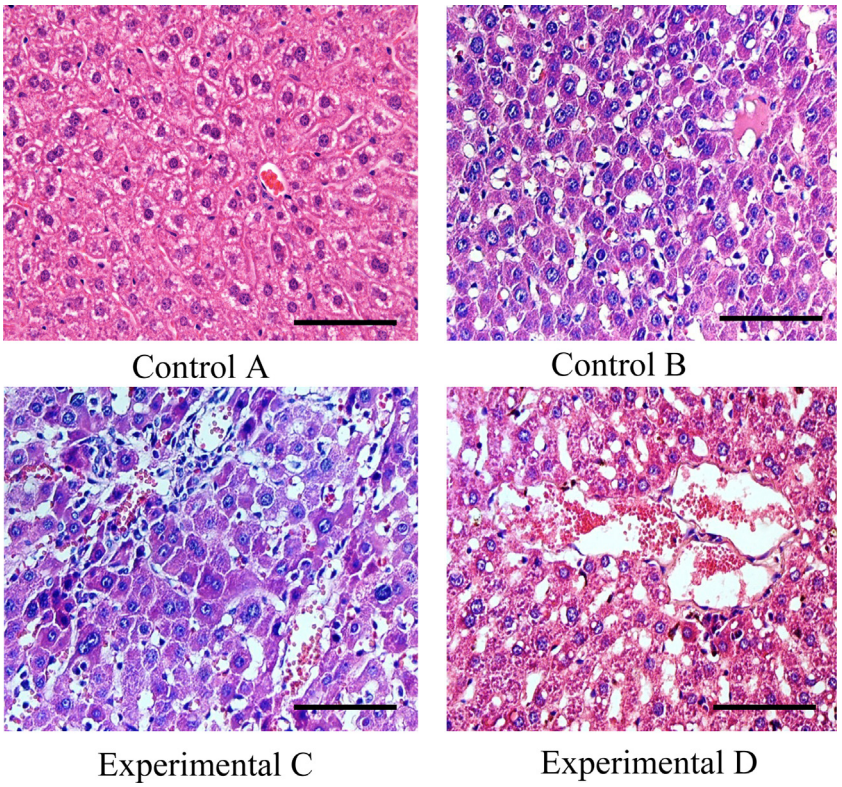

Fig. 5. Effect of imidacloprid on histology of liver. Hematoxylin and eosin staining (scale bar 20×) shows normal liver morphology in adult mice (A, B) that were maintained on a normal diet. Experimental images (C, D) indicate imidacloprid treated high dose group (15 mg/kg/day, for 15 days) showing presence of erythrocytes that appear to be hypertrophied blood vessels and mild dilation of sinusoidal spaces.

and vegetables puts consumers especially children on the risk in a cumulative manner [1]. Previous studies of IC acute toxicity following occupational, accidental or suicidal ingestion indicated mild clinical effects such as tachycardia, nausea, vomiting to severe respiratory failure, seizures and even death in human [19,20]. Toxicological studies of imidacloprid are limited, but they have shown mild pathological changes in the brain, kidney and liver of exposed rats at high doses [11]. Imidacloprid acts in insects at the nAChRs, suggesting that this may also be targeted in mammals [10]. There have been reports those patients with clinical toxicity due to exposure of imidacloprid in a deliberate suicide attempt [21–24].

In the present study, we have investigated possible effects of imidacloprid on mouse body weight, liver functions and kidney. Our result shows that 15 days oral exposure of high dose of imidacloprid to mice has produced significant toxic effects. It is of interest to know that there was a significant reduction in the body weight of high dose exposed mice. The decrease in body weight is inconsistent with the previous studies [11,25]. Badgujar et al. stated that the high exposure of imidacloprid resulted in decreased body weight and other pathological changes in BALB/c mice [26]. Avery et al. [27] and Werner et al. [28] reported that the reduction in intake of food may be due to repellent effects of the pesticides, whereas Li et al. [29] reported that food intake reduction could be attributed to the toxic effects of pesticides that made the endangered animal, less food intake and resulted in the loss of body weight. High doses of imidacloprid resulted in reduced body weight and the liver were identified as the principal target organ for toxicity [30].

Due to the toxic potential of imidacloprid results in increased liver weight of high dose exposed animals with concomitantly increased in the activity of serum GOT and GPT. A significant increase in serum LFTs levels in mice with high dose exposure of imidacloprid was observed when compared with the low dose treated animals. The results of our study are inconsistent with the earlier reports [11,31,32]. It may be due to degeneration and necrosis of hepatocytes, which attributes an increased permeability of the cell membrane that results in the release of transaminases into the blood stream. These findings were correlated with the histopathological and ultrastructural changes observed in the liver of mice. Previous research suggests that the imidacloprid can cause oxidative stress and inflammation in organs like liver and brain in rats [33]. Our findings are complementary with Bhardwaj et al., who reported that mild focal necrosis of the liver and hepatocellular damage following a subchronic imidacloprid exposure in female rats [11]. The data of the present study have indicated that imidacloprid induced liver toxicological effects in mice at 15 mg/kg/day dose level when exposed for a period of 15 days. Ultimately, in this study, the liver of mice exposed to the high imidacloprid treatment evidenced congestion and fatty degeneration. Such

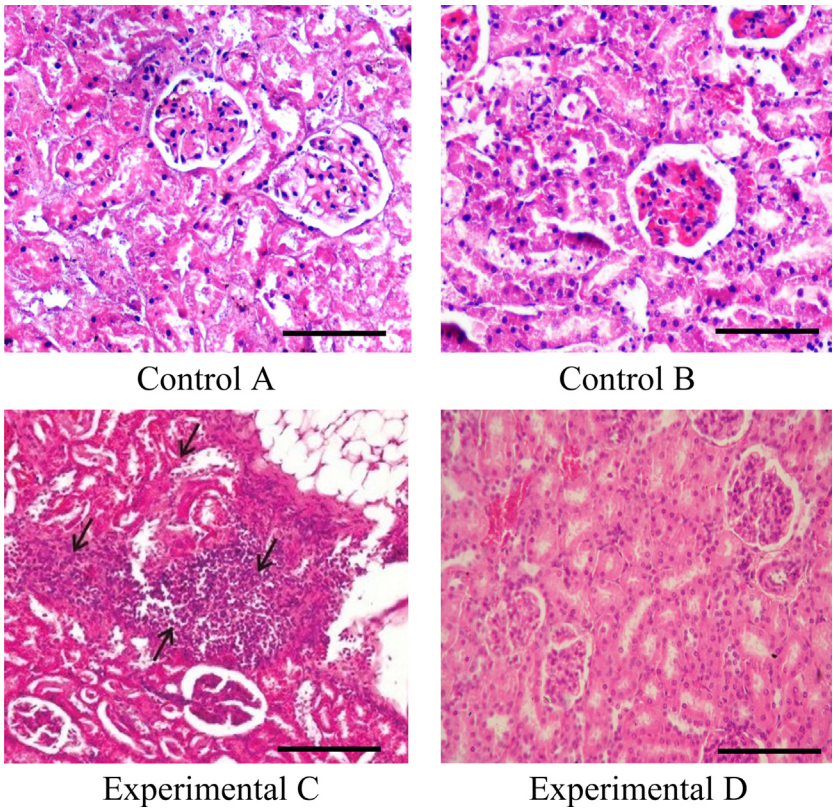

Fig. 6. Effect of imidacloprid on histology of kidney. Hematoxylin and eosin staining (scale bar 40×), no histopathologic changes were seen among control group (A, B). In experimental group mice (C, D) indicate mild congestion in the glumeroli, and vacuolar degeneration of tubular cells with arrows showing areas of inflammation and hemolysis in experimental groups at a dose of (15 mg/kg/day).

outcomes are highly suggestive of mild-to-moderate hepatotoxic effects of this insecticide. These findings are in agreement with Kammon and colleagues [16]. The hepatotoxic effects noted here are in agreement with the findings of soujanya et al. [25].

Bhardwaj and co-workers studied the imidacloprid nephrotoxicity and found tubular changes in the kidney and its increased weight at higher dose exposed rats [11]. We also found higher doses of imidacloprid increased the weight of kidney with tubular changes shows its nephrotoxicity in mice. The kidneys showed hemorrhages, vacuolar degeneration of tubular epithelial cells as well as focal coagulative necrosis. However, the kidneys, the major detoxification organs for many xenobiotics, are frequently susceptible to the nephrotoxic effects. Hisopathological examination revealed lesions in kidney tissues produced by imidacloprid. Similar signs of toxicity were also reported earlier by Kammon and colleagues [16] and [30].

Creatinine is derived mainly from the catabolism of creatine found in muscle tissue and its catabolism to creatinine occurs at a steady rate. Severe kidney damage will lead to increased creatinine levels. In the present study, serum creatinine and BUN showed marginal increase in the experimental group in comparison to control animals and increase relates to renal failure. Serum creatinine and blood urea nitrogen (BUN) determine the glomerular filtration rate (GFR) improperly in renal failure. Serum creatinine and BUN have the potential to be a more precise marker for GFR. Similar results were reported in earlier studies in rats [11,34].

Our study shows that the imidacloprid has induced toxicological effects on mice at 15 mg/kg/day dose level when exposed for a period of 15 days. However, 5 and 10 mg/kg/day doses have no adverse effects. Thus, on the basis of analysis parameters such as, organ body weight ratio, biochemical, urine analysis and histopathology examination of experimental mice, it may be suggested that the IC dose of 5 and 10 mg/kg/day did not produced any detrimental effects. Therefore, these doses considered as no observed adverse effect level (NOAEL) as compared with 15 mg/kg/day as a lowest observable effect level (LOEL) to mice.

5. Conclusion

Dose-dependent decrease in body weight increased LFTs indicative of degenerative changes in the liver and tubular changes in the kidney were caused by imidacloprid. Besides, imidacloprid caused slight degeneration of the tubules and glomeruli of the kidney at the dose of 15 mg/kg/day. It is concluded that imidacloprid can impair the liver function and kidney at a dose much lower than the LD_{50} in mice, but at the doses up to 10 mg/kg body weight, does not significantly affect the liver function and kidney.

Conflict of interest statement

There are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.toxrep.2014.08.004.

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