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RESEARCH ARTICLE
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Preliminary acute toxicity study on imidacloprid in Swiss albino mice

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Abstract

Aim: To ascertain the maximum tolerated dose (MTD) and to investigate the acute oral toxic effects of imidacloprid towards Swiss albino male mice.

Materials and Methods: The MTD of imidacloprid was determined in pilot dose range finding study following the standard method. Animals were observed for toxic signs and symptoms after oral administration of MTD of imidacloprid in single dose. The body weights of animals were recorded on alternate day. Animals were sacrificed on 14th day and changes in hematological parameters (Hb, TEC, TLC and DLC) and morphometric measurements (length, breadth, thickness and weight) of various body organs (heart, liver, spleen, kidney, testis and epididymis) were examined. The student's t-test was applied to statistically analyze the results.

Results: The MTD of imidacloprid was determined to be 110 mg/kg body weight. The sign and symptoms of acute toxicity were ataxia, rigidity and fasciculation of muscles, protrusion of eye ball and tremors of head. Imidacloprid treatment resulted in decreased body weight gain as compared to the control group. The changes in hematological parameters were not significant between imidacloprid treated and control groups. Also the values of relative organ weights and morphometric measurements of various body organs did not differ significantly between the control and imidacloprid treated animals.

Conclusions: MTD of imidacloprid in Swiss albino male mice through oral route was determined for the first time. Study revealed a non-toxic effect of imidacloprid on body weight, relative organs weight, hematological parameters and morphometric measurements of various body organs in mice.

Keywords: hematology, imidacloprid, morphometric measurement, MTD, toxicity

Introduction

Contamination of natural resources by indiscriminate and hysterical use of pesticides is potential threat to animal and human health. Imidacloprid (IMI) is most widely used neonicotinoids insecticide in agriculture with registration for use on over 140 crops in over 120 countries [1]. Such large scale use can exaggerate the toxic properties and adverse effects of insecticide and can be fatal for human as well as animal health. The study of acute toxicity of IMI 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 [2, 3]. The analysis of toxic properties of drugs and chemicals using *in vivo* mammalian systems (mice or rat) is of enormous value which reflects indirect toxic effects on humans because of their high degree of presumptive human relevance. Since IMI is now being considered as replacement for other available pesticides, relative benefits and risk must be considered.

Determination of maximum tolerated dose (MTD) or median lethal dose (LD_{50}) is imperative for acute toxicity study of insecticides. The LD_{50} of IMI in mice is reported to be 131 mg/kg body weight (BW) [4, 5]. The toxic effect of IMI on hematological and morphometric parameters of different body organs in swiss albino mice is not known.

Thus in present study, the acute toxicity of IMI at MTD level was studied in swiss albino male mice following oral administration. Subsequently, hematological changes and toxic effects on morphometric parameters of heart, liver, kidney, spleen, epididymis and testis were studied.

Materials and Methods

Chemicals and experimental animals: IMI (technical grade, >98% purity) originally obtained from Indofil Chemicals Company, Mumbai, India was used in the present investigation. Swiss albino mice weighing between 17-27 g were procured from Disease Free Small Animal House, LLRUVAS, Hisar. The mice were acclimatized to laboratory conditions for 2-3 days before the experiments were conducted. The temperature of animal house was maintained between 22 - 27°C throughout the experiment.

Ethical approval: The prior approval of Institutional Animal Ethical Committee was obtained for use of the animals in this study.

Table-1. Oral doses of imidacloprid (IMI) used for determination of maximum tolerated dose (MTD) in Swiss albino mice

Dose (mg/kg; orally)	Number of mice died/number of mice administered	Percent mortality
260	1/4	25
200	1/5	20
170	1/5	20
140	1/5	20
110	0/10	0
100	0/4	0

MTD of imidacloprid: 110 mg/kg BW orally

Determination of MTD: Various doses of IMI with respect to oral LD_{50} (131 mg/kg) [5] were screened for determination of MTD in pilot dose range finding study following standard method [6]. These doses were presented in Table-1. Briefly, the study was conducted in small groups of mice (n=2/3/5) using several doses including few lethal doses. Animals in each group were administered single oral dose through gavage and thereafter several iterations were conducted. The MTD was selected that produced clear observable signs of toxicity without resulting in lethality. The MTD was then verified in a larger group of animals (n=10) which were observed for 14 days for grossly observable behavioral effects.

Acute toxicity study: The acute toxicity study for testing of chemicals was performed as described by earlier workers [7]. Following administration of IMI at MTD level on first day, the body weights of all animals in all groups were recorded on alternate day for 14 days. The animals were sacrificed on 14th day and blood was taken directly from heart using heparinized hypodermic syringe and collected in test tube rinsed with heparin saline solution (5 mg heparin/ml NSS). Hemoglobin (Hb), total erythrocytes count (TEC) and total leukocytes count (TLC) were estimated [8, 9]. The blood smears were fixed with methanol for five min, stained with Giemsa's stain (1:10 dilution) for 25 min and observed under oil immersion lens for differential leukocytes count (DLC).

Necropsies were performed on sacrificed animals and morphometric measurements (length, breadth, thickness and weight) of various body organs *viz.* heart, liver, kidney, spleen, epididymis and testis was recorded. In case of liver, major lobe was taken for length, breadth and thickness. The relative organ weights were calculated using formula: organ weight (g)/body weight (g) X 100.

Statistical analysis: The student's t-test using SPSS statistics 17.0 software (IBM Corporation, New York, USA) was applied to statistically analyze the results obtained with different study groups.

Results and Discussion

MTD finding experiment and clinical profile: Following the pilot dose range finding study, the MTD of IMI in Swiss albino mice was found to be 110 mg/kg BW by oral route. To the best of our knowledge, the present study is the first to demonstrate that the MTD of IMI in Swiss albino male mice through oral route is 110 mg/kg BW. which is much lower when compared to the previously reported LD_{50} (131 mg/kg) [5]. However, MTD determined in the present study is much higher when compared to the MTD (50 mg/kg BW) in female BALB/c mice reported earlier [10] which may be due to variations in drug metabolism rate of two strains. Further elucidation of genetic basis of strain specific pesticide metabolism and toleration underlies identification of potentially susceptible human or animal genotype. The huge difference in MTD of IMI in present study can be correlated with the earlier reports of variations in the gender, strains and species specific detoxification processes [11-14].

The oral exposure of IMI at MTD level resulted in appearance of gross observable toxicity signs within 10-15 min of administration. The pesticide produced mild clinical signs with nervous manifestations. Tremors of head region started episodically after 12-15 min (lasting for 2 sec to 1 min) with peak level after 20-22 min of administration. Body posture was abnormal with hind limbs abducted from body. The severity of all clinical signs and symptoms decreased with the time and disappeared within 24 hours. The mice manifested the prominent abdominal respiration at slow rate in initial stages which later on became normal. Administration of higher dose of IMI was associated with the increased severity and earlier onset of toxicity symptoms. Animals introduced with the pesticide at dose higher than MTD exhibited very high respiratory rate just before death. The occurrence of tremors, convulsions and high respiratory rate correlate with the agonist nature of IMI at nicotinic acetylcholine receptors (nAChR) which induces neuromuscular paralysis [15]. The rapid appearance (within 10-15 min of administration) and disappearance (within 24 hours of administration) of toxic signs and symptoms correlate with the earlier reports of prompt absorption (92-95% within 1 hour) and excretion of more than 90% within 24 hours of administration of IMI [16, 17]. The absence of toxic sign and symptoms after 24 hours indicated no delayed toxic effect of IMI.

Effect of Imidacloprid treatment on body weights of mice: Following administration of IMI at MTD dose level on day 1, the body weights of mice were recorded on alternate day for 14-days and are presented in Table-2. A transient decrease in BW gain was observed in IMI treated animals compared to control group. The body growth was found to be slightly decreased as inferred from the transient decrease in BW which is reported to be a common symptom of IMI treated animals [18].

Table-2. Effect of imidacloprid treatment on body weight (g) of mice

Treatment	Days post IMI administration							
	0	2	4	6	8	10	12	14
C (n=5)	20±0.5	20.6±0.4	22.2±0.5	22.6±0.4	23.2±0.6	23.8±0.5	24.2±0.6	24.8±0.5
IMI (n=10)	23.5±0.5	24.1±0.5	24.5±0.7	25.5±0.9	26.1±0.9	26.1±1.1	26.8±1.0	26.9±0.9

Values are mean body weight in grams (mean ± SE); C: control (300 mg/kg BW); IMI: imidacloprid (110 mg/kg BW)

Table-3. Effect of imidacloprid treatment on various hematological parameters in mice

Treatment	Hb(g/dl)	TEC(x10^6/cmm)	TLC(x10^3/cmm)	DLC (no. of cells/100 leukocyte)				
				N	L	M	B	E
C (n=5)	11.58±0.39	11.67±0.97	11.67±1.57	7.11±2.28	86.81±3.70	3.61±1.23	1.70±0.58	0.41±0.19
IMI (n=10)	11.23±0.39	9.75±0.82	14.32±1.10	5.01±0.42	90.78±1.10	3.28±0.77	0.67±0.22	0.28±0.12
t_{cal}	NS	NS	NS	NS	NS	NS	NS	NS

Values are Mean ± SE; NS: Not Significant; C: control (300 mg/kg BW); IMI: imidacloprid (110 mg/kg BW); Hb: Hemoglobin; TEC: Total Erythrocyte Count; TLC: Total Leukocyte Count; DLC: Differential Leukocyte Count; N: Neutrophils; L: Lymphocyte; M: Monocyte; B: Basophils; E: Eosinophils

Table-4. Effect of imidacloprid treatment on relative organ weights (g/100g b.wt) of mice

Treatment	Heart	Liver	Spleen	Kidney		Testis		Epididymis	
				Right	Left	Right	Left	Right	Left
C (n=5)	0.49±0.02	5.70±0.50	0.56± 0.07	0.64±0.03	0.64±0.04	0.39±0.01	0.39±0.02	0.14±0.01	0.14±0.01
IMI (n=10)	0.53±0.02	5.50±0.34	0.75± 0.08	0.72±0.03	0.74±0.03	0.40±0.02	0.39±0.02	0.16±0.01	0.16±0.01
t_{cal}	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values are relative organ weights in gram/100g BW. (Mean ± SE) calculated using formula- organ weight (g)/body weight (g) X 100); C: control (300 mg/kg BW); IMI: imidacloprid (110 mg/kg BW); NS: Not Significant

Table-5. Effect of imidacloprid treatment on morphometric measurements

Treatment	Heart	Liver	Spleen	Kidney		Testis		Epididymis	
				Right	Left	Right	Left	Right	Left
Length in mm/100g body weight									
C (n=5)	24.9±1.1	47.4±2.4	68.0±3.7	35.1±1.2	36.0±0.8	25.1±0.7	25.0±0.5	61.7±2.7	57.3±3.0
IMI (n=10)	26.4±0.9	44.2±2.0	69.2±3.7	33.0±1.1	35.7±1.2	25.6±1.1	25.4±1.0	60.2±2.4	62.5±2.2
t_{cal}	NS	NS	NS	NS	NS	NS	NS	NS	NS
Breadth in mm/100g body weight									
C (n=5)	16.9±0.1	88.9±4.9	16.8±1.4	18.6±0.7	21.1±0.4	16.2±0.4	15.8±0.5	-	-
IMI (n=10)	16.1±0.6	77.1±3.9	17.1±0.7	18.7±0.6	19.2±0.7	14.7±0.6	16.7±0.6	-	-
t_{cal}	NS	NS	NS	NS	NS	NS	NS	-	-
Thickness in mm/100g body weight									
C (n=5)	19.4±1.0	13.7±0.8	9.3±0.6	17.9±0.5	18.1±1.0	14.4±0.8	15.3±0.9	-	-
IMI (n=10)	16.9±0.6	12.2±0.9	9.8±1.3	16.6±0.7	16.0±0.9	13.5±0.7	12.6±1.2	-	-
t_{cal}	NS	NS	NS	NS	NS	NS	NS	NS	NS

Values are mean ± SE; n_1=5 and n_2=10; NS: Not Significant; C: control (300 mg/kg BW); IMI: imidacloprid (110 mg/kg BW)

Acute toxic effects of imidacloprid on various hematological parameters of mice: The results of hematological studies are presented in Table-3. No significant changes in the hematological parameters (Hb, TEC, TLC, differential neutrophilic and lymphocytic count) were found in IMI treated animals when compared to the control group. The mean values of Hb and TEC were decreased non-significantly in IMI treated animals. However, the mean values of TLC were increased non-significantly, which might be due to rebound effect of IMI on haemopoietic tissues as suggested earlier [19]. The non toxic nature of aqueous leaf extracts of *Ficus exasperata* has been described earlier based on its effects on hematological parameters (WBC count, platelet and hemoglobin estimation), body weight and temperature [20].

Effect of imidacloprid treatment on morphometric measurements and relative organ weights: The weights of various body organs are presented in Table-4. The mean values of relative organ weights of different organs *viz.* heart, liver, spleen, right kidney, left kidney, right testis, left testis, right epididymis and left epididymis did not differ significantly between the control and IMI treated groups. 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 in terms of its length, breadth and thickness. The values of morphometric measurements of various vital organs were measured and presented in Table-5. No significant difference was observed in the values of length, breadth and thickness of heart, liver, spleen, kidney, testis and epididymis of IMI treated animals as compared to the control group. The changes in morphometric parameters of body organ relates with the toxic properties of chemicals. Acute toxicity study has been described earlier in terms of changes in certain morphometric parameters viz surface volume and organs volume [21, 22]. However, reports with the same morphometric parameters

(length, breadth & thickness) of organs as used in the present study was lacking in literature.

The non significant changes in the values of BW gain, hematological parameters, morphometric measurements and relative organ weights indicated that exposure of IMI at MTD level has no toxic effects on hematological system and weights and morphology of body organs. Further, delayed toxic effects were also absent in the experimental animal. Several researchers have considered IMI as safer than organophosphorus and other toxic insecticides used in agriculture based on median lethal dose and severity of the poisoning symptoms [23, 24, 25]. The present study also reveals the high MTD of IMI and non toxic effects on body growth and hematopoietic system. The substitution of IMI for organophosphorus compounds may be promoted after considering the relative benefits and hazards with other used pesticides; however more research is required on IMI with respect to its toxic potential on genomic, metabolic, physiologic and developmental processes.

Conclusion

The MTD of IMI in Swiss albino mice through oral route was determined for the first time. The MTD in Swiss albino mice was more than twice the reported MTD in BALB/c mice reflecting the existence of strain specific variations in pesticide metabolism. The present study also revealed that IMI at MTD level have no adverse delayed toxic effects on hematological parameters and growth profile (weights and morphometric parameters) of various body organs.

Authors' contributions

VK conceptualized the aim of the study, designed the experiment, supervised dosing regimen and statistical. PB executed the experiments, conducted statistical analyses and drafted the manuscript. JSP and AKS helped in analyses, and drafted the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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