Toxicology International, *Vol 27(3&4), 86-92, 2020, DOI: 10.18311/ti/2020/v27i3&4/22489*

Original Research
ISSN (Print): 0971-6580
ISSN (Online): 0976-5131

Toxic Effects of Sub-Chronic Oral Exposure of Imidacloprid on Biochemical Parameters in Buffalo Calves

Tsewang Dorjay, Rajdeep Kaur*, S. P. S. Saini and H. S. Sandhu

Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana – 141 004, Punjab, India;
rajksidhu@yahoo.com

Abstract

Imidacloprid, is a highly effective and widely used neonicotinoid insecticide for crop protection and veterinary pest control, which has gained a major market control in the past several years. The present investigation was undertaken to evaluate the toxic potential of imidacloprid in buffalo calves when administered for prolonged periods. Evaluation of various biochemical markers of hepatic, renal and muscular injury was done using standardized procedures. Oral administration of imidacloprid (@ 0.5 mg/kg/day; 90 days) in male buffalo calves, produced varying degrees of nasal discharge, lachrymal discharge, lethargy and weakness. Imidacloprid exposure caused a significant increase in the levels of plasma aspartate aminotransferase (12.3%), alanine aminotransferase (172.2%), lactate dehydrogenase (13.7%), gamma-glutamyltranspeptidase (57.5%), alkaline phosphatase (37.2%), acid phosphatase (220.0%), blood urea nitrogen (148.4%), creatinine (5.22%) and cholesterol (68.9%). However, no significant changes were observed in the enzymatic activity of creatine kinase, plasma cholinesterase as well as total protein and glucose levels.

Keywords: Biochemical, Buffalo Calves, Imidacloprid, Subchronic

1. Introduction

Imidacloprid is a systemic, chloro-nicotinyl insecticide with soil, seed and foliar uses[1]. It was introduced for commercial use in the last decade and is being increasingly used worldwide on rice, cereals, maize, potatoes, vegetables, sugar beets, fruits, cotton, hops and turf. Imidacloprid produces its action by interfering with the transmission of stimuli in nervous system of insect. Specifically, it causes a blockage of nicotinergic pathway that is more abundant in insects as compared to warm-blooded animals, thereby making it selectively more toxic to insects than warm-blooded animals. This blockage leads to the accumulation of acetylcholine, an important neurotransmitter, resulting in paralysis of the insect and eventually death. Imidacloprid is effective as contact and via stomach action[2].

Since being introduced in the insecticide market in 1992, the use of imidacloprid has increased immensely. For the most part, it is replacing the acetylcholinesterase inhibitors, the organophosphorus compounds and methylcarbamates, which display higher mammalian toxicity and decreased effectiveness due to pest resistance. In mammals, the acute neurotoxicity of imidacloprid is largely due

**Author for correspondence*

to action at the α4β2 and the homomeric α7 receptors in the brain[3–4]. The CNS effects cause excessive stimulation, convulsions and seizures followed by depression and coma. The action of imidacloprid in the brain is very complex due to its biotransformation to a large number of metabolites with varying degree of stability and toxicity. One of these metabolites, desnitro-imidacloprid is of particular interest, because it has a nicotinic-type action that prefers mammalian versus insect nicotinic actelycholinereceptors[5]. Its animal toxicity is similar to that of the parent compound nicotine, exhibited as fatigue, twitching cramp and weakness leading to asphyxia. In animals, salivation and vomiting have been reported following oral exposure whereas high doses of oral exposures may lead to lethargy, vomiting, diarrhea, salivation, muscle weakness and ataxia which are all indicative of imidacloprid's action on nicotinicreceptors[6]. Other signs of exposure at high doses included uncoordinated gait, tremors and reduced activity[7].

The unchecked indiscriminate usage of pesticides has been correlated with several un-explained health problems in both humans and animals and there are strong indications that pesticides are associated with high incidences of cancer, hormonal imbalance, reproductive problems, neurological derangements, etc. The potential hazard due to pesticide residues on the health of livestock and long term exposure of animals to these chemicals is a growing concern and requires further investigations. Therefore, the present investigation was undertaken in buffalo calves to evaluate basic biochemical parameters as markers of toxicological insult produced by long term exposure of imidacloprid.

2. Material and Methods

The experiments were performed on eight healthy male buffalo calves of 6-12 months age and weighing between 60-120 kg, procured from the University Dairy Farm and local market. The animals were acclimatized in the animal shed of department under uniform conditions for 2 weeks, prior to the commencement of experiment. During this period, all animals were subjected to regular clinical examination and treated with anthelmintics for deworming. The animals were maintained on green fodder and wheat straw and water was provided *ad libitum*. The permission to conduct the experiment was duly taken from the University Animal Ethics Committee vide Memo No. VMC/2010/IAEC/1976-92 dated 17.12.2010.

The animals were randomly divided into 2 groups viz. 1 and 2 for the study. Animals of Group 1 were kept as healthy control whereas animals of Group 2 were orally administered imidacloprid at a dose rate of 0.5 mg/kg/day for 90 consecutive days. Blood samples were collected in heparinized vials via jugular venipuncture of experimental animals on days 0, 10, 20, 30, 40, 50, 60, 70, 80 and 90 of treatment and on 100th and 110th day post-treatment. The biochemical parameters namely Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Gamma-Glutamyltranspeptidase (GGT), Lactate Dehydrogenase (LDH), Creatine Kinase (CK), Alkaline Phosphatase (ALP), Acid Phosphatase (ACP), plasma cholinesterase, total proteins, Blood Urea Nitrogen (BUN), blood glucose, creatinine and cholesterol were assayed with semi automatic biochemical analyser using autopack kits procured from Bayer (Siemens, Medical Solutions Diagnostics Ltd, Baroda). Data were subjected to one-way analysis of variance and Duncan's multiple range test for comparing the means to find effect between treatment groups using SPSS® 14.0 software.

3. Results and Discussion

Daily oral administration of imidacloprid at the dose rate of 0.5 mg/kg/day for 90 consecutive days produced varying degrees of nasal and lachrymal discharge, lethargy and weakness, however, the treated animals recovered within 20 days of the post-treatment period. Imidacloprid (@ 0.5 mg/kg/day) produced significant elevation in the level of plasma aspartate (12.3%) and alanine aminotransferase (172.2%), as compared to the control group (Table 1). The increase in the activity of these enzymes might be attributed to liver dysfunction and disturbance in their synthesis. ALT is employed as a marker of hepatocellular damage in a variety

of species and the increased plasma aminotransferase activity in the present study could be due to cellular damage or increased plasma membrane permeability Bayer (Siemens, Medical Solutions Diagnostics Ltd, Baroda). These observations are in agreement with Balani et al[9], Bhardwaj et al[10], Kaur et al[11] and Siddiqui[12] who reported a similar trendin male white Leghorn (WLH) chicks, rats, cow calves and cockerels, respectively.

A significant elevation in the level of plasma lactate dehydrogenase, to the extent of 13.7%, was observed after long-term oral administration of Imidacloprid (Table 1). Any cellular damage to liver, lung, muscle, kidney and heart release this enzyme into systemic circulation[13]. Similarly, a significant increase in the level of GGT (57.5%), which is found in many tissues but the primary source is liver, especially the cell membrane near the bile caniliculi, was observed in the present study (Table 1). Although the exact cause of increased GGT level in this investigation could not be ascertained, yet cholestic disorders in all species are associated with increased GGT activity[14]. The use of plasma reference values help to interpret the variation of serum GGT mainly in hepatobiliary diseases of cattle, sheep, goats and cholestic disorders of dogs.

Long-term oral administration of imidacloprid caused significant incline in plasma alkaline (37.2%) and acid-phosphatase (220.0%) activity, as depicted in Table 2. ALP is found in a number of body tissues including liver; thus, the plasma ALP activity is not specific for the liver. Release of ALP also occurs due to its increased synthesis brought about by a variety of liver conditions[15] whereas the acid phosphatase activity in blood is derived from cell lysosomes in bone, prostate, platelets, erythrocytes and spleen[16]. A similar increase in plasma alkaline phosphatase levels was alsoreported by Kaur et al[11] in cow calves after repeated oral administration of imidacloprid.

Oral administration of imidacloprid produced no significant change in the levels of plasma creatine kinase (Table 2). The present findings are however not in agreement with Friedman et al[17] who reported elevations in CK in patients after acute exposure to pesticides. Similarly, no significant change was observed in the activity of plasma cholinesterase in the present study (Table 2). The present findings are in tune with Kaur et al[11] who reported thatimidacloprid after repeated oral administration in cow calves produced no significant effects on plasma cholinesterase enzyme.

Imidalcoprid (@ 0.5 mg/kg/day) produced significant elevation of blood urea nitrogen and creatinine, to the extent of 148.4% and 5.22%, respectively, as compared to the control (Table 3). The enhanced levels of BUN are related to kidney disease, urinary obstruction or enhanced protein catabolism[18]. In addition, the increase in plasma urea and creatinine concentrations in the present experiment is indicative of a certain degree of kidney dysfunction and some damage to the renal clearance pathway. On the other hand, no significant change in the levels of total proteins and blood glucose was observed in imidacloprid treated group (Table 3). Although liver plays an important role in protein metabolism yet total protein concentration is of little value in assessment of liver function[19]. Similar findings have been earlier reported by Balani et al[9] and Kaur et al[11] in male White Leghorn (WLH) chicks and cow calves, respectively. Imidacloprid administration produced significant elevation in the level of plasma cholesterol (68.9%) [Table 3]. There are number of factors that results in hypercholestrolemia, including increased production of cholesterol by hepatocytes or enterocytes, decreased intravascular lipolysis or processing of lipoproteins and defective hepatic uptake of low density lipoproteins. Cholesterol is esterified in liver and is used for the synthesis of bile acids and steroid hormones. Esterification is depressed in liver diseases and hypercholestrolemia is seen in animals with bile duct occlusion.

Therefore, on the basis of the present investigation it can be suggested that imidacloprid is a low-risk insecticide which does not produce marked alterations in the various hepatic, renal and muscular biomarkers on long term oral exposure and thus can be safely used in the recommended concentrations in buffalo calves.

Table 1. Effect of repeated oral administration of imidacloprid (@ 0.5 mg/kg/day; 90 days) on plasma aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and gamma glutamyltranspeptidase in buffalo calves

	Treatment										Post-treatment	
Days	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**10**	**20**
	Aspartate aminotransferase (U/L)											
Control	155.5 ± 3.71^{a}	141.8 ± 2.68^{a}	146.5 ± 6.36^{a}	139.3 ± 6.84^{a}	138.4 ± 6.77^{a}	125.7 ± 4.25^{a}	129.7 ± 1.84^{a}	119.2 ± 6.54^{a}	146.0 ± 8.33^{a}	142.0 ± 9.60^{a}	146.7 ± 9.04^{a}	131.2 ± 8.86^{a}
Imidacloprid	162.7 ± 3.30^{a}	171.0 ± 4.12^{bc}	180.7 ± 5.34^{b}	167.7 ± 5.72^{b}	182.7 ± 9.77^{ab}	166.0 ± 5.35^{a}	141.2 ± 6.75^{a}	138.7 ± 7.21^{a}	157.0 ± 5.92^{a}	159.2 ± 9.52^{a}	165.5 ± 8.29^{a}	136.7 ± 6.66^{a}
	Alanine aminotransferase (U/L)											
Control	27.3 ± 1.11^{a}	31.3 ± 1.11^{a}	43.8 ± 4.92^{a}	48.5 ± 3.59^{a}	48.0 ± 4.02^{ab}	41.5 ± 1.50^{a}	39.5 ± 1.71^{a}	37.0 ± 2.08^{a}	49.8 ± 1.65^{a}	44.8 ± 4.73^{a}	41.8 ± 2.36^{a}	35.5 ± 2.10^{a}
Imidacloprid	27.6 ± 1.26^{a}	29.0 ± 1.78^{a}	50.3 ± 3.27^{a}	44.0 ± 3.72^{a}	49.5 ± 2.98^{ab}	41.5 ± 1.85^{a}	45.0 ± 3.19^{ab}	46.0 ± 2.83^{ab}	75.0 ± 2.34^{bc}	68.3 ± 2.72^{c}	64.3 ± 2.29^{c}	35.0 ± 1.29^{a}
	Lactate dehydrogenase (U/L)											
Control	1255.7 ± 56.1^{a}	1230.7 ± 78.3^{a}	1329.5 ± 45.6^{a}	1313.5 ± 25.3^{a}	1417.5 ± 45.1^{a}	1255.0 ± 79.1^{a}	1106.7 ± 20.8^{a}	915.5 ± 76.7^{a}	1082.0 ± 63.2^{a}	1139.5 ± 103.9^{a}	1136.0 ± 36.6^{a}	1156.7 ± 65.3^{a}
Imidacloprid	1320.7 ± 15.9^{a}	1434.2 ± 49.8^{b}	1395.2 ± 45.6^{ab}	1387.2 ± 19.1^{ab}	1492.5 ± 49.7^{a}	1446.7± 56.5^{b}	1399.2 ± 40.7^{b}	1346.5 ± 115.8^{b}	1411.2 ± 48.2^{b}	1502.2 ± 107.9^{b}	1445.0 ± 100.9^{b}	1438.2 ± 118.0^{b}
	Gamma glutamyl transpeptidase (U/L)											
Control	9.75 ± 0.63^{a}	10.0 ± 0.91^{a}	11.3 ± 0.75^{a}	11.0 ± 0.91^{ab}	11.5 ± 0.64^{a}	10.3 ± 0.85^{a}	10.3 ± 0.85^{a}	9.50 ± 0.64^{a}	9.00 ± 0.91^{a}	10.5 ± 0.95^{a}	8.75 ± 0.95^{a}	8.25 ± 0.85^{a}
Imidacloprid	10.0 ± 0.40^{a}	12.3 ± 1.03^{ab}	13.0 ± 0.91^{a}	13.5 ± 0.50^{c}	15.8 ± 0.85^{b}	12.3 ± 1.03^{a}	11.5 ± 0.50^{ab}	10.3 ± 1.03^{a}	10.5 ± 1.23^{a}	15.0 ± 1.68^{b}	9.25 ± 0.48^{a}	9.50 ± 0.87^{a}

Values given are expressed as U/L and represents the Mean ±SE of 4 animals.
Values lacking a common superscript in a given column differs significantly from each other ($p<0.05$).

Table 2. Effect of repeated oral administration of imidacloprid (@ 0.5 mg/kg/day; 90 days) on plasma alkaline phosphatase, acid phosphatase, creatine kinase and acetylcholinesterase in buffalo calves

	Treatment										Post-treatment	
Days	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**10**	**20**
	Alkaline phosphatase (U/L)											
Control	104.3 ± 1.93^{a}	110.0 ± 7.49^{a}	114.0 ± 5.76^{a}	110.7 ± 6.42^{a}	110.0 ± 5.87^{ab}	119.0 ± 1.47^{a}	101.0 ± 6.87^{a}	102.2 ± 6.06^{a}	109.7 ± 5.72^{a}	111.5 ± 8.78^{a}	104.3 ± 4.85^{a}	113.5 ± 3.77^{a}
Imidacloprid	104.3 ± 2.75^{a}	107.7 ± 4.01^{a}	108.7 ± 4.90^{a}	124.7 ± 8.46^{a}	121.2 ± 7.56^{b}	123.7 ± 8.79^{a}	98.50 ± 6.59^{a}	117.0 ± 1.47^{b}	142.7 ± 7.33^{b}	143.0 ± 5.61^{b}	121.0 ± 2.74^{b}	115.5 ± 6.40^{a}
	Acid phosphatase (U/L)											
Control	1.00 ± 0.00^{a}	1.75 ± 0.10^{a}	2.25 ± 0.25^{a}	2.25 ± 0.25^{ab}	2.25 ± 0.25^{a}	2.00 ± 0.00^{a}	2.50 ± 0.29^{a}	2.50 ± 0.29^{a}	2.50 ± 0.29^{a}	2.25 ± 0.25^{a}	2.00 ± 0.00^{ab}	2.00 ± 0.00^{a}
Imidacloprid	1.25 ± 0.10^{a}	1.75 ± 0.10^{a}	2.25 ± 0.25^{a}	2.00 ± 0.00^{a}	2.00 ± 0.00^{a}	1.75 ± 0.25^{a}	6.75 ± 0.48^{b}	4.00 ± 0.00^{b}	3.75 ± 0.25^{b}	3.00 ± 0.41^{a}	2.25 ± 0.25^{b}	2.50 ± 0.29^{a}
	Creatine Kinase (U/L)											
Control	184.5 ± 3.40^{a}	184.7 ± 2.78^{ab}	193.7 ± 1.25^{a}	190.2 ± 1.70^{a}	166.2 ± 7.07^{ab}	197.2 ± 4.77^{a}	168.7 ± 4.73^{a}	163.0 ± 3.08^{a}	159.5 ± 5.51^{a}	183.0 ± 2.97^{a}	160.2 ± 7.71^{a}	169.7 ± 4.09^{a}
Imidacloprid	190.5 ± 1.32^{a}	178.7 ± 1.44^{a}	190.7 ± 8.77^{a}	184.2 ± 3.45^{a}	183.5 ± 2.36^{b}	198.7 ± 4.52^{a}	157.0 ± 8.09^{a}	161.0 ± 3.34^{a}	177.7 ± 7.91^{a}	175.2 ± 10.39^{a}	163.0 ± 3.49^{a}	178.2 ± 4.90^{a}
	Acetylcholinesterase (U/L)											
Control	180.8 ± 6.81^{a}	180.9 ± 4.16^{a}	180.3 ± 3.84^{a}	185.4 ± 2.72^{a}	186.7 ± 3.39^{a}	180.0 ± 12.9^{a}	180.0 ± 1.13^{a}	185.1 ± 6.56^{ab}	198.9 ± 4.08^{a}	174.9 ± 4.26^{ab}	181.2 ± 1.07^{ab}	183.4 ± 4.95^{a}
Imidacloprid	180.7 ± 4.92^{a}	191.7 ± 3.20^{a}	190.5 ± 4.35^{a}	184.0 ± 1.27^{a}	199.9 ± 2.18^{a}	202.5 ± 2.31^{a}	181.1 ± 1.42^{a}	195.4 ± 2.04^{b}	207.9 ± 8.63^{a}	188.0 ± 4.66^{b}	189.3 ± 3.73^{b}	197.9 ± 9.02^{a}

Values given are expressed as U/L and represents the Mean ±SE of 4 animals.
Values lacking a common superscript in a given column differs significantly from each other ($p<0.05$).

Table 3. Effect of repeated oral administration of imidacloprid (@ 0.5 mg/kg/day; 90 days) on blood urea nitrogen, creatinine, total protein, cholesterol and blood glucose in buffalo calves

	Treatment										Post-treatment	
Days	**0**	**10**	**20**	**30**	**40**	**50**	**60**	**70**	**80**	**90**	**10**	**20**
	Blood Urea Nitrogen											
Control	7.00 ± 0.71^{a}	6.25 ± 0.48^{a}	6.50 ± 0.64^{a}	9.75 ± 0.85^{a}	9.50 ± 0.29^{a}	6.25 ± 0.48^{a}	6.50 ± 0.64^{a}	9.75 ± 0.48^{a}	6.75 ± 0.48^{ab}	7.50 ± 0.64^{a}	5.55 ± 0.25^{ab}	6.00 ± 0.41^{a}
Imidacloprid	7.75 ± 0.48^{a}	10.8 ± 0.48^{b}	11.3 ± 0.75^{b}	10.3 ± 1.43^{a}	19.3 ± 0.85^{d}	10.8 ± 0.85^{b}	7.25 ± 0.48^{a}	13.8 ± 0.75^{b}	5.50 ± 0.29^{a}	7.75 ± 0.49^{a}	6.75 ± 0.48^{b}	6.50 ± 0.29^{a}
	Creatinine											
Control	1.03 ± 0.02^{a}	1.33 ± 0.15^{a}	1.33 ± 0.06^{ab}	1.35 ± 0.10^{a}	1.35 ± 0.10^{a}	1.21 ± 0.18^{a}	0.95 ± 0.04^{a}	0.90 ± 0.06^{a}	0.96 ± 0.02^{a}	1.00 ± 0.04^{a}	1.14± 0.11^{a}	1.01 ± 0.08^{a}
Imidacloprid	1.15 ± 0.07^{a}	1.32 ± 0.03a	1.18 ± 0.06a	1.31 ± 0.04^{a}	1.12 ± 0.08^{a}	1.07 ± 0.07^{a}	1.21 ± 0.03^{b}	0.88 ± 0.06^{a}	0.97 ± 0.06^{a}	0.99 ± 0.05^{a}	1.17 ± 0.04^{a}	0.96 ± 0.02^{a}
	Total protein											
Control	6.47 ± 0.02^{a}	6.62 ± 0.06^{a}	6.68 ± 0.28^{a}	6.79 ± 0.24^{a}	6.48 ± 0.16^{a}	6.69 ± 0.14^{a}	6.41 ± 0.20^{a}	6.51 ± 0.11^{a}	6.72 ± 0.11^{ab}	6.39 ± 0.14^{a}	6.39 ± 0.12^{a}	6.56 ± 0.14^{a}
Imidacloprid	6.65 ± 0.16^{a}	6.54 ± 0.12^{a}	6.48 ± 0.25^{a}	6.86 ± 0.11^{a}	6.80 ± 0.05^{a}	6.77 ± 0.17^{a}	6.99 ± 0.09^{a}	6.69 ± 0.12^{a}	7.04 ± 0.18^{b}	6.39 ± 0.24^{a}	6.66 ± 0.14^{a}	6.53 ± 0.14^{a}
	Cholestrol											
Control	43.3 ± 2.35^{a}	46.3 ± 3.12^{a}	46.0 ± 2.34^{a}	62.8 ± 0.95^{a}	62.0 ± 2.74^{ab}	68.3 ± 2.93^{a}	65.8 ± 2.21^{a}	65.3 ± 2.06^{a}	61.8 ± 2.09^{a}	63.8 ± 2.43^{a}	64.3 ± 3.75^{a}	54.8 ± 2.29^{a}
Imidacloprid	49.0 ± 1.59^{a}	53.3 ± 2.49^{ab}	46.0 ± 2.94^{a}	82.8 ± 1.93^{b}	53.0 ± 5.21^{a}	65.0 ± 2.41^{a}	68.5 ± 2.90^{a}	59.8 ± 1.84^{a}	67.0 ± 4.74^{a}	74.3 ± 0.85^{b}	66.5 ± 1.55^{a}	54.8 ± 2.49^{a}
	Glucose											
Control	50.0 ± 0.73^{a}	56.6 ± 0.62^{a}	60.2 ± 1.04^{a}	59.5 ± 0.82^{ab}	52.9 ± 2.09^{ab}	51.5 ± 3.35^{a}	56.3 ± 1.86^{ab}	56.9 ± 1.99^{a}	48.5 ± 1.01^{a}	58.2 ± 1.79^{a}	57.8 ± 2.01^{a}	54.9 ± 1.58^{a}
Imidacloprid	51.9 ± 1.57^{a}	52.9 ± 2.37^{a}	52.8 ± 4.76^{a}	62.4 ± 1.79^{b}	59.6 ± 2.94^{b}	49.7 ± 2.68^{a}	49.9 ± 2.78^{a}	48.8 ± 3.06^{a}	45.3 ± 3.38^{a}	52.9 ± 3.53^{a}	53.4 ± 1.69^{a}	47.9 ± 2.72^{a}

Values given are expressed as U/L and represents the Mean ±SE of 4 animals.
Values lacking a common superscript in a given column differs significantly from each other ($p<0.05$).

4. Acknowledgement

The financial support provided by the Guru Angad Dev Veterinary and Animal Sciences University is highly appreciated.

5. References

1. Quintas G, Armenta S, Garrigues S, Guardia MDL. Fourier transform infrared determination of imidacloprid in pesticide formulations. Journal of the Brazilian Chemical Society. 2004; 15(2):307–12. https://doi.org/10.1590/S0103-50532004000200023
2. Kidd H, James D. Agrochemicals Handbook. Third Ed. Cambridge, England: Royal Society of Chemistry; 1994.
3. Tomizawa M, Cowan A, Casida JE. Analgesic and toxic effects of neonicotinoid insecticides in mice. Toxicology and Applied Pharmacology. 2001; 177:77–83. https://doi.org/10.1006/taap.2001.9292
4. Shimomura M, Okuda H, Matsuda K, Komai K, Akamatsu M, Sattelle DB. Effects of mutations of a glutamine residue in loop D of the alpha7 nicotinic acetylcholine receptor on agonist profiles for neonicotinoid insecticides and related ligands. British Journal of Pharmacology. 2002; 137:162–9. https://doi.org/10.1038/sj.bjp.0704848
5. Tomizawa M, Casida JE. Imidacloprid, thiacloprid and their imine derivatives upregulate the alpha 4 beta 2 nicotinic acetylcholine receptor in M10 cells. Toxicology and Applied Pharmacology. 2000; 169:114–20. https://doi.org/10.1006/taap.2000.9057
6. Wismer TN. Insecticides. Clinical Veterinary Toxicology; Plumlee, K. H., Ed.; St. Louis: Mosby; 2004. p. 184-85.
7. Thyssen J, Machemer L. Imidacloprid: Toxicology and Metabolism. Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptor; Yamamoto I, Casida J. E. Eds.; Springer-Verlag: Tokyo; 1999; 9:213–22. https://doi.org/10.1007/978-4-431-67933-2_9
8. Reagan WJ, Yang RZ, Park S, Goldstein R, Brees D, Gong DW. Metabolic adaptive ALT isoenzyme response in livers of C57/BL6 mice treated with dexamethasone. Toxicol Pathol. 2012; 40(8):1117–27. https://doi.org/10.1177/0192623312447550
9. Balani T, Agrawal S, Thaker AM. Hematological and biochemical changes due to short-term oral administration of imidacloprid. Toxicology International. 2011; 18(1):2–4. PMid: 21430911. https://doi.org/10.4103/0971-6580.75843
10. Bhardwaj S, Srivastava MK, Kapoor U, Srivastava LP. 90 days oral toxicity of imidacloprid in female rats: Morphological, biochemical and histopathological evaluations. Food and Chemical Toxicology. 2010; 48(5):1185–90. PMid: 20146932. https://doi.org/10.1016/j.fct.2010.02.009
11. Kaur B, Sandhu HS, Kaur R. Toxic effects of subacute oral exposure of imidacloprid on biochemical parameters in crossbred cow calves. Toxicology International. 2006; 13(1):43–7.
12. Siddiqui MA. Toxicological and immunological studies of sub acute exposure of cockerels to imidacloprid and quinalphos. [M.V. Sc. Thesis]. Anand: Gujarat Agricultural University; 2004.
13. Katarzyna A, Hryniewicz-Jankowska A, Renata T. Lactate dehydrogenase 5: An old friend and a new hope in the war on cancer. Cancer Letters. 2015; 358(1): 1–7. https://doi.org/10.1016/j.canlet.2014.12.035
14. James EE, Elizabeth CW. Association of γ-glutamyl transferase (GGT) activity with treatment and clinical outcomes in chronic hepatitis C (HCV). Hepatology. 2013; 57(5):1725–33. PMid: 23258530. https://doi.org/10.1002/hep.26203
15. Nicole JF, BeverlyAK. Alkaline phosphatase: Beyond the liver. Veterinary Clinical Pathology. 2007; 36(3):223–33. https://doi.org/10.1111/j.1939-165X.2007.tb00216.x
16. Rosol TJ, Capen CC. Calcium regulating hormones and diseases of abnormal mineral (Calcium, Phosphorus and Magnesium) metabolism. Kaneko J J, Harvey J W and Bruss M L (ed). Clinical Biochemistry of Animals. 5th edn. San Diego: Academic Press; 1997. p. 619–702. https://doi.org/10.1016/B978-012396305-5/50024-5
17. Friedman LS, Brautbar N, Barach P, Wolfe A, Richter ED. Creatine phosphate kinase elevations signaling muscle damage following exposures to anticholinesterases: 2 sentinel patients. Archives of Environmental Health. 2003; 58(3):167–71. PMid: 14535577. https://doi.org/10.3200/AEOH.58.3.167-171
18. Finco DR. Kidney function. Kaneko J J (ed). Clinical Biochemistry of Domestic Animals. 5th edn. San Diego: Academic Press; 1989. p. 447–84.
19. Brar RS, Sandhu HS, Singh A. Veterinary clinical diagnosis by laboratory methods. New Delhi: Kalyani Publishers, Ludhiana; 2000.