The Pharma Innovation Journal 2023; 12(5): 4561-4566

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(5): 4561-4566

www.thepharmajournal.com
Received: 08-02-2023
Accepted: 12-03-2023

Debolina Dattaray
Department of Veterinary Pharmacology and Toxicology, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Tushar Kanta Garnaik
Department of Veterinary Pharmacology and Toxicology, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Raja Loganathan
Department of Veterinary Pharmacology and Toxicology, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Tapan Kumar Mandal
Department of Veterinary Pharmacology and Toxicology, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Tapas Kumar Sar
Department of Veterinary Pharmacology and Toxicology, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Manisha Chhikara
Central Insecticides Laboratory, Ministry of Agriculture and Farmers Welfare, Faridabad, Haryana, India

Corresponding Author:
Raja Loganathan
Department of Veterinary Pharmacology and Toxicology, West Bengal University of Animal and Fishery Sciences, Kolkata, West Bengal, India

Effects of subacute exposure of imidacloprid on haemato-biochemical parameters in black Bengal goat (*Capra hircus*)

Debolina Dattaray, Tushar Kanta Garnaik, Raja Loganathan, Tapan Kumar Mandal, Tapas Kumar Sar and Manisha Chhikara

Abstract
Imidacloprid is a member of the neonicotinoids insecticide group and the systemic accumulation in food acts as a major source of exposure. The indubitable need for investigations of imidacloprid toxicity in non-target species is required to understand the implications on food chain and ultimately human health. In the present study, we aimed to investigate the subacute effect of imidacloprid on haemato-biochemical parameters of black Bengal goats. In this experiment, 20 healthy black Bengal goats were divided into two groups, Group A: 4 goats and group B:16 goats further subdivided into groups I (DI) and II (DII)of 8 animals each which were administered technical grade imidacloprid as oral gavage for 28 days at minimum toxic dose (45 mg/kg bw/d) and maximum non-toxic dose (25 mg/kg bw/d). Out of 8 animals, 4 were sacrificed on the 29th day of the experiment, and the remaining 4 were sacrificed at the end of the withdrawal period of 15 days. Blood and serum samples were collected on days 0, 14th, and 28th day respectively, and hemogram, and biochemical parameters were observed. No significant change in the hemogram but slight increase in serum protein, AST, and ALT levels observed which could be corroborated with the tissue biochemical levels and pathological changes in the liver and kidney tissue. The findings of the study were somewhat similar to the previously documented literature and contradicted at a few parameters. Thus, the results of the experiment indicate that imidacloprid had a minimum toxic effect at the given dose.

Keywords: Imidacloprid, *Capra hircus*, haematology, serum biochemistry, insecticide

1. Introduction
Pesticides are any substances or mixture of substances intended for attracting, preventing, destroying, or mitigating any pest. They are mainly applied in agriculture to protect crops from insects, weeds, and bacterial or fungal diseases during growth and to protect foods during storage from rats, mice, insects or diverse biological contaminants (Bolognesi *et al*., 2011) [1]. They can also be used as plant growth regulators, defoliant and desiccant and nitrogen stabilizers. Besides the plant safety concern, pesticides also pose a threat to various non- target species. The residues of the undesirable contaminants in animal feed and food products have become a serious threat to public health. Pesticides may enter the body by different ways depending on species, metabolic peculiarities, and susceptibility to toxins (Lushchak *et al*., 2018) [2].

Imidacloprid [1-(6-chloro-3-pyridylmethyl)-2-nitroimino-imidazolidine] (IMI) belongs to a major new class of synthetic insecticides called neonicotinoids and is used widely to control insect pests on crops and fleas on domestic animals. Imidacloprid acts as a potent agonist on insect nicotinic acetylcholine receptors (nAChRs), specifically at the α-subunits of the nicotinic receptor, like nicotine (Matsuda *et al*., 2001) [3]. The receptors nAChRs are ligand-gated ion channels involved in synaptic transmission in the central nervous system (CNS) (Dani *et al*., 2001) [4]. Neonicotinoids, including IMI are comparatively more toxic to insects than to mammals, thus providing an excellent example of selective toxicity (Matsuda *et al*., 2001) [3]. The selective toxicity of IMI on insects than to mammals is attributed to differences in their binding affinity or potency in the nicotinic acetylcholine receptor (Bal *et al*., 2010) [5]. IMI doses are moderately toxic. It damages the biological nervous system through calcium ion imbalance, mitochondrial dysfunction, oxidative stress, and DNA damage, ultimately leading to biological death. The acute oral LD50 for rats and mice is 450 mg/kg bw and 150 mg/kg bw, respectively. In male rats, the NOEL dose of IMI is 14 mg/kg bw/d.

Chronic exposure to IMI is associated with neurobehavioral deficits in offspring rats following in utero exposure, genotoxic and mutagenic alterations (Bal *et al*., 2012) [6]. Behavioural and respiratory distress signs, disturbances of motility, narrowed palpebral fissures, transient trembling, and spasms were noted in rats and mice treated orally at doses >200 mg/kg bw and >71 mg/kg bw, respectively. In the recent years Imidacloprid has been reported as an emerging contaminant in all parts of the world and has the potential to adversely impact ecosystems and human health (Pang *et al*., 2020) [7]. Therefore, imidacloprid was taken into consideration to study the toxic effects of this insecticide.

According to the 20th Livestock Census conducted during October 2018, the total Goat population in the country in 2019 is 148.88 Million showing an increase of 10.14% over the previous census, contributing to about 27.8% of the total livestock population (Vikaspedia, 2022) [8]. The milk production of the goat is 5.75 million metric ton according to the 2017 financial year data. Goat milk contributes about 3% of total milk production in India. Milk-producing animals accumulate pesticides from contaminated feed and by inhaling contaminated air. Goats have very high exposure to pesticide-treated fields as they graze in an open field. Considering the above, black Bengal goats were selected as the test animal of the study.

2. Materials and Methods

2.1 Experimental Design

Technical grade imidacloprid was obtained from M/S United Phosphorus Limited, Ankleshwer, Gujarat, India and the purity was more than 97.3%. Clinically healthy 20 black Bengal female goats weighing 8-12 kg of approximately 1½ - 2 years old were used for this experiment. Prior permission and approval from the Institutional Animal Ethics Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) were taken before conducting the experimental protocol, and the animals were kept in standard laboratory conditions. The goats were divided into two groups consisting of 4 goats in A group and 16 goats in the group B. Group A consisted of 4 animals, while 16 animals of group B were further divided into groups I and II with 8 animals, which were administered with a minimum toxic dose (45 mg/kg) and maximum non-toxic dose (25 mg/kg) dissolved in 0.5% DMSO daily orally for 28 days. Out of 8 animals, 4 were sacrificed on the 29th day of the experiment, and the remaining 4 were sacrificed at the end of the withdrawal period of 15 days. Haemogram, blood biochemical, blood residue, and the hormonal status during the experiment in black Bengal goats following daily oral administration at two dose levels of pesticides for 28 days was observed as a part of the study.

2.2 Sample collection

2.2.1 Blood: Blood samples were collected from the jugular vein of each animal of all the groups at the 0th, 14^{th,} and 28th day of the experiment. Blood samples were collected in EDTA vials for hematological study, residue analysis, and blood biochemical study, and in sodium fluoride for glucose estimation, 2 mL of blood was allowed to clot for separation of serum and stored at –20˚C for AST, ALT, serum protein and hormonal assay and other biochemical parameters. Blood for oral disposition kinetic study was collected from right jugular venipuncture at different time intervals of 0, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 36, 48, and 72 h following oral administration of pesticides in goats.

2.2.2 Tissue: Four animals of all the groups were sacrificed on 29th day of the experiment and the remaining four animals on day 15 post-dosing (after the withdrawal period) *i.e*., on 43rd day. Organs like liver, kidney, heart, lung, skeletal muscle, spleen, fat, adrenal gland, brain, uterus, bile, skin, large intestine, small intestine, rumen, reticulum, omasum, abomasum, bone, mammary gland, urinary bladder, and subscapular lymph node were collected for estimation of residue of imidacloprid pesticide. A small portion of liver, heart and kidney were collected for estimation of tissue biochemical parameters and a part of the caudate lobe of liver was used to estimate cytochrome P_{450}. Tissue homogenate 10% and 5% was used to estimate various parameters like catalase, total protein, AST and ALT, etc. For hormone estimation, a male goat was introduced into the herd before starting the experiment to bring synchronization of estrous among the female goats (Shelton, 1960; Skinner *et al*., 1969) [9, 10]

2.3 Analytical Procedures

The methods described in Schalm's veterinary haematology (Jain, 1986) [11] were used for the estimation of haemoglobin, total erythrocyte count (TEC), packed cell volume (PCV), total leukocyte count (TLC), and differential leukocyte count (DLC). For Serum protein, Bi-Uret method (Wooton, 1974) [12] was used. Blood glucose was determined by the Glucose Assay kit by GOD-POD method using *in vitro* Diagnostic kit (Trinder, 1969) [13]. Serum alanine and aspartate aminotransferase activities were measured using COGENT kit based on 2, 4-DNPH method (Reitman, 1957) [14]. The estimation of lipid peroxidation was done (Buege 1978) [15]. For blood biochemical parameters, haemolysate (5%) was prepared and performed following haemoglobin (Coffin, 1953) [16] superoxide dismutase (Misra *et al*., 1972) [17], and catalase (Aebi, 1974) [18], Reduced glutathione (Griffith, 1980) [19] methods. A portion of liver, kidney, heart, spleen, and lymph node collected from both control and experimental goats and fixed in 10% formal-saline for histopathological study (Lillie *et al*., 1976) [20].

2.4 Statistical analysis

The results were expressed as Mean ± Standard error (SE). The data were analyzed statistically using one way analysis of variance (ANOVA) between the groups in SPSS 10.0 version of the software.

3. Results and Discussion

Blood parameters are considered important for assessing the body's homeostasis in mammals. These parameters provide an opportunity to determine the presence of toxic metabolites and their constituents in the body (Etim *et al*., 2014) [21]. Figure 1 reveals a significant reduction in case of hemoglobin. Non-significant reduction in TEC and PCV values in the groups intoxicated imidacloprid was also evident. A similar finding has been reported by Américo-Pinheiro *et al*. (2019) [22] where a significant decrease with in the number of red blood cells was observed in the fish compared to those not exposed to the insecticide. Such changes in the red blood cells were suggestive of affecting oxygen transport and can be related to the inflammation process of gill lamellae caused by poisoning (Vosylienė, 1999) [23].

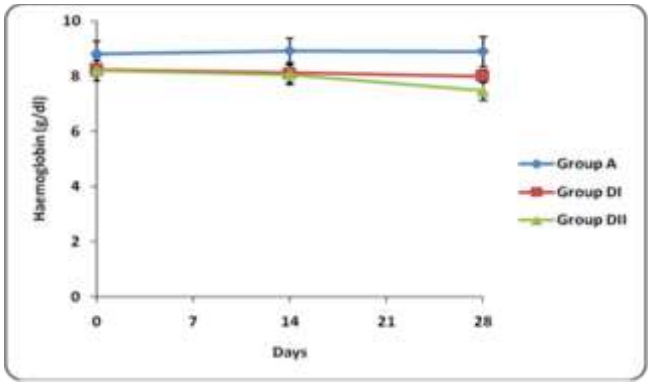

Fig 1: Effect of imidacloprid on haemoglobin (g/dl) in goats following daily oral administration at two dose levels (25 and 45 mg/kg BW) for 28 days. (Mean±SE of 4 replicates).

On the other hand, no significant changes were established in the hematological parameters like Hb and TEC, in acute toxicity of imidacloprid on swiss albino mice (Bagri *et al*., 2013) [24]. Findings of a study also reported that Tilapia fish exposed to sublethal concentration of IMID-polluted water significantly reduced hemoglobin, MCHC, Eosinocytes, and monocytes counts (Naiel *et al*., 2020) [25]. Whereas, in contrary to our findings transient elevation and decrease in the PCV and Hb concentrations was noted at various hourly intervals in South American grayish baywing (*Agelaioides badius*) after IMI treatment.

Findings from another study reported a significant decrease in TEC, PCV, Hb, MCV, MCH, MCHC, and increase in TLC in 30 mg/kg bw imidacloprid treated group (Soujanya *et al*., 2020) [26]. Significant decrease in number of erythrocytes, hemoglobin (Hb), hematocrit (HCT) and erythrocyte sedimentation rate (ESR) in a dose dependent manner on exposure to IMI in case of female mice was also documented (Kataria *et al*., 2016) [27]. Meanwhile, a report of the decrease in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration in adult male wistar rats that received an IMI suspension via the oral route at doses of 1.5, 5, and 15 mg/kg bw for 45 consecutive days was observed (Tonietto *et al*., 2022) [28]. The various factors that may be responsible for reduction in Hb/RBC includes internal haemorrhage, decrease in Hb synthesis or due to an increase in Hb destruction and due to defective maturational and functional status of different marrow cell lineage. The pesticide metabolites may damage the supportive stromal matrix due to which adequate number of hematopoetic cells may not form or hematopoetic process halts. Severe oxidative stress can also lead to hemolysis as erythrocyte is very sensitive to peroxidative reactions, which are normally protected by catalase and glutathione activity. Moreover, the histopathological sections of the liver and kidney indicated hemorrhages and hyperemia that correlate well with the hematological changes.

Table 1 reveals a non-significant reduction in TLC and DLC. Our results were somewhat different than that as reported by a researcher where oral exposure of imidacloprid for 28 days on birds resulted in a significant reduction in TLC (Balani *et al*., 2011) [29]. Reports of significant increase in neutrophil count, and significant decrease in lymphocyte count in imidacloprid treated male albino rats were also seen Soujanya *et al* (2020) [26]. They further stated that Imidacloprid is a ring-structured compound, and compounds having benzene ring or other ring structures act as a hapten that combines with the protein constituent of leukocytes to form an antigen to which the animal develops antibodies that are toxic to leukocytes, causing either lysis or agglutination (Benjamin, 1978) [30] and this may have caused leukocytopenia.

Table 1: Effect of imidacloprid on leukocytic series of goats following daily oral administration at two dose levels (25 and 45 mg/kg bw) for 28 days.

Parameters	Groups \ Days	0	14	28
TLC (x10^9 /L)	A	14.47±0.94	15.64±1.02	14.66±1.60
	DI	14.57±1.00	14.00±1.07	14.04±0.90
	DII	16.96±0.80	16.11 ±0.90	15.34±0.91
Lymphocytes (%)	A	66.90±1.81	68.07±1.25	68.75±1.36
	DI	65.05±1.47	64.87±1.28	63.83±1.32
	DII	66.77±1.96	63.77±1.31	58.39±1.63
Eosinophils (%)	A	10.55±0.82	10.20±1.13	10.37±0.78
	DI	10.63±0.61	10.05±0.84	9.11±0.77
	DII	10.28±0.85	9.10±0.64	8.47±0.55
Neutrophils (%)	A	48.25±1.54	49.43±1.90	49.79±1.74
	DI	47.76±1.75	46.94±1.65	46.66±1.60
	DII	55.78±1.70	52.44±1.14	49.28±0.86

Group A; control group administered with DMSO
Group DI and DII were administered orally with 25 and 45 mg/kg bw imidacloprid for 28 days.

A significant increase in total leukocytes, neutrophils, and lymphocytes in tilapia fish exposed to all sub-lethal concentrations of imidacloprid was documented (Américo-Pinheiro *et al*., 2019) [22]. The justification was that increasing the number of leukocytes will assist in removing cell debris and necrotic tissue and stimulate the immune defense of the host (John, 2007) [31]. The non-significant reduction was also observed in hematological parameters like (Hb, TEC, differential neutrophilic and lymphocytic count) of IMI treated swiss albino mice. However contradictory to our results, the mean value of TLC had increased non-significantly, which might be a rebound effect of imidacloprid on hematopoetic tissue (Bagri *et al*., 2013) [24]. Pesticide-contaminated feed administered to rabbits has also shown a non-significant change in the case of TLC. However, the percentage of neutrophils and eosinophils had significantly decreased in the pesticide-exposed rabbit (Kobir *et al*., 2020) [32].

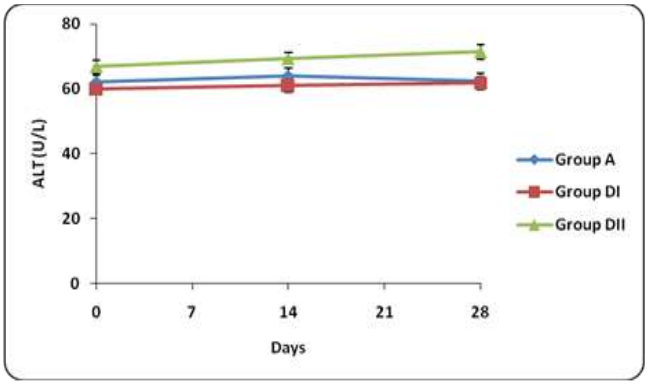

Fig 2: Effect of imidacloprid on serum ALT (U/L) in goats following daily oral administration at two dose levels (25 and 45 mg/kg bw) for 28 days. (Mean±SE of 4 replicates)

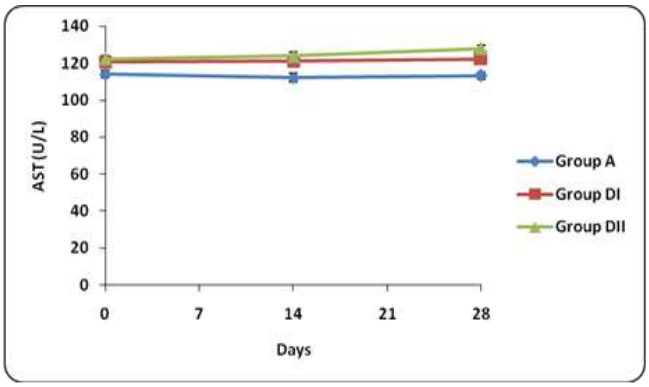

Fig 3: Effect of imidacloprid on serum AST (U/L) in goats following daily oral administration at two dose levels (25 and 45 mg/kg bw) for 28 days. (Mean±SE of 4 replicates)

The results of our serum biochemical parameters revealed non- significant induction in the serum protein level. Contrary to our results Kumar *et al*. (2014) [33] reported a significant decrease in total protein on IMI exposure (37.5, 75, and 112.5 mg/kg bw) in the case of female albino mice. Documentation of significant reduction in total protein in imidacloprid (80 mg/kg bw) exposed rats for 28 days orally was also found (Soujanya *et al*., 2013) [34]. Liver is considered the target organ of insecticide toxicity. Group DII showed a non-significant (P<0.05) increase in ALT values on the 28th day of the experiment compared to 0th day. In the case of AST value, Group DII defined a non-significant (P<0.05) difference with the increase in their values on the 14th, and 28th days of the experiment compared to the 0th day value. Our findings indicated in Figure 2, and Figure 3 were in agreement with (Vohra *et al*., 2014) [35] where reports of significant increase in liver marker enzymes such as AST, ALT, and phosphatases on imidacloprid intoxicated rats were seen, which indicated increased permeability, damage and necrosis of hepatocytes (Sathiavelu *et al*., 2009) [36]. It may be due to hepatocyte degeneration, which contributed to increased cell membrane permeability that resulted in the release of transaminases into the bloodstream. In case of liver injury, the transport function of the hepatocytes is disturbed, which leads to leakage of the plasma membrane, thereby causing an increased enzyme level in serum (Jadon *et al*., 2007) [37]. The activity of serum enzymes like AST, ALT, ALP, urea and GGT were significantly increased after 28 days of exposure to fipronil-IMI mixture in case of male albino rats in a 28 day study (Badawy *et al*., 2018) [38]. Qadir *et al*. (2014) [39] reported significant increase in ALT and AST levels on four days of treatment of imidacloprid (120mg/l) in the case of Labeo rohita. Similar findings were also documented in Arfat *et al*., 2014 [40], where a high dose of imidacloprid caused a significant elevation of serum biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate kinase (SGPT), alkaline phosphatase (ALP), and total bilirubin (TBIL). The results were in conjunction with the liver and kidney histology, which indicated hepatotoxicity and nephrotoxicity at a high dose of imidacloprid @15 mg/kg/day in mice.

In the case of tissue protein, no significant difference was observed in the protein values of liver, heart and kidney between groups A, DI, and DII. In the case of Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), no significant difference was observed in ALT values of liver, heart, and kidney between groups A, DI, and DII. But in the case of AST value only in the heart, the value increased significantly (P<0.05) in group DII compared to group A. Our results corroborated with the findings (Toor *et al*., 2013) [41], where treatment of rats with imidacloprid caused changes in the enzyme levels of aspartate aminotransferase (AST) and alkaline phosphatase (AKP) in liver tissue by causing a significant increase in AST level in the liver tissue. The increased level of enzymes suggests the detoxification of pesticides, which may be based on mutation of genes responsible for synthesizing these enzymes. A significant increase in AST and ALT was also reported (Mahajan *et al*., 2018) [42] in the imidacloprid treated rats, where the animals received combined treatment of imidacloprid along with arsenic. The increased levels of these cellular enzymes could be due to degeneration and necrosis of hepatocytes, resulting in increased cell membrane permeability with leakage of these biomarkers into the blood.

4. Conclusions

Imidacloprid is a member of the neonicotinoids insecticide group. It acts as a selective agonist on insect nicotinic acetylcholine receptors. The systemic accumulation in food acts as a major source of imidacloprid exposure. The indubitable need for investigations of imidacloprid toxicity in non-target species, we evaluated the effects of 28-day oral exposure of minimum toxic dose (45mg/kg bw) and maximum non-toxic dose (25 mg/kg bw) of imidacloprid in female black Bengal goats. The findings of our study were somewhat similar to the previously documented literature and contradicted at a few parameters. The imidacloprid exposure revealed no significant change in the hemogram of intoxicated animals. The blood biochemical changes were evident with an slight increase in serum protein, AST, and ALT levels which could be corroborated with the findings of tissue biochemical levels and pathological changes in the liver and kidney tissue. Thus, the results of the experiment indicate that imidacloprid had a minimum toxic effect at the given dose.

5. References

1. Bolognesi C, Merlo FD. Pesticides: human health effects. Encyclopedia of Environmental Health. 2011;16:438-453.
2. Lushchak VI, Matviishyn TM, Husak VV, Storey JM, Storey KB. Pesticide toxicity: A mechanistic approach. EXCLI Journal. 2018;8(17):1101-1136.

DOI: 10.17179/excli2018-1710.
3. Matsuda K, Buckingham SD, Kleier D, Rauh JJ, Grauso M, Sattelle DB. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. Trends in Pharmacological Sciences 2001;22(11):573-580. DOI: 10.1016/s0165-6147(00)01820-4.
4. Dani JA, De Biasi M. Cellular mechanisms of nicotine addiction. Pharmacology Biochemistry and Behaviour. 2001;70(4):439-446. DOI: 10.1016/s0091-3057(01)00652-9.
5. Bal R, Erdogan S, Theophilidis G, Baydas G, Naziroglu M. Assessing the effects of the neonicotinoid insecticide imidacloprid in the cholinergic synapses of the stellate cells of the mouse cochlear nucleus using whole-cell patch-clamp recording. Neurotoxicology. 2010;31(1):113-120. DOI: 10.1016/j.neuro.2009.10.004.
6. Bal R, Türk G, Tuzcu M, Yilmaz O, Kuloglu T, Gundogdu R, *et al*. Assessment of imidacloprid toxicity on reproductive organ system of adult male rats. Journal of Environmental Science and Health, Part B 2012;47(5):434-444. DOI: 10.1080/03601234.2012.663311.
7. Pang S, Lin Z, Zhang Y, Zhang W, Alansary N, Mishra S, *et al*. Insights into the Toxicity and Degradation Mechanisms of Imidacloprid Via Physicochemical and Microbial Approaches. Toxics. 2020;8(3):65. DOI: 10.3390/toxics8030065.
8. Vikaspedia; c2022 [Weblink: https://vikaspedia.in/agriculture/agri-directory/reports-and-policy-briefs/20th-livestock-census] [Visited on 20 May, 2023].
9. Shelton M. Influence of the presence of a male goat on the initiation of estrous cycling and ovulation of Angora does. Journal of Animal Science. 1960;19(2):368-375.
10. Skinner JD and Hofmeyr HS. Effect of the male goat and of progesterone and PMSG treatment on the incidence of oestrous in the anoestrous Boer goat doe. Proceedings of South African Society of Animal Production. 1969;8:155-156.
11. Jain NC. Schalm's Veterinary Hematology. 4th Edn. Lea & Febiger; c1986.
12. Wooton IDP. Estimation of protein by Bi-uret method. Microanalysis in Medical Biochemistry. 5th Ed, Edinburgh: Churchill Livingstone; c1974. p. 156-158.
13. Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. Annals of clinical Biochemistry. 1969;6(1):24-27.
14. Reitman S and Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology. 1957;28 (1):56-63.
15. Buege JA. The thiobarbituric acid assay. Methods in Enzymology. 1978;52:306-307.
16. Coffin DL. Manual of Veterinary Clinical Pathology. 3rd Edn. Comstock publishing company. Inc. Ithaca. New York; c1953.
17. Misra HP and Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry. 1972;247(10):3170-3175.
18. Aebi H. Methods of enzymatic analysis, ed. New York, Academic Press. 1974;2:674.
19. Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. Analytical Biochemistry. 1980;106(1):207-212.
20. Lillie RD and Fullmer HM. Histopathologic Technic and Practical Histochemistry. 4th edn. McGraw-Hill. London, UK; c1976.
21. Etim NN, Williams ME, Akpabio U, Offiong EEA. Haematological parameters and factors affecting their values. Agricultural Science. 2014;2(1):37-47.
22. Américo-Pinheiro JHP, da Cruz C, Aguiar MM. Sublethal Effects of Imidacloprid in Hematological Parameters of Tilapia (Oreochromis niloticus). Water, Air & Soil Pollution. 2019;230(193):1-7. DOI: 10.1007/s11270-019-4256-0
23. Vosylienė MZ. The effect of heavy metals on haematological indices of fish (survey). Acta Zoologica Lituanica. 1999;9(2):76-82.
24. Bagri P, Kumar V, Sikka AK, Punia JS. Preliminary acute toxicity study on Imidacloprid in Swiss albino mice. Veterinary World. 2013;6(12):955-959. DOI: 10.14202/vetworld.2013.955-959
25. Naiel MAE, Ismael NEM, Abd El-hameed SAA, Amer MS. The antioxidative and immunity roles of chitosan nanoparticle and vitamin C-supplemented diets against imidacloprid toxicity on *Oreochromis niloticus*. Aquaculture. 2020;523:735219.
26. Soujanya S, Lakshman SM, Madhuri D, Reddy AG, Rama Rao SV. Hematological Alterations Induced by Imidacloprid and Ameliorative Effect of Withania somnifera in Female Albino Wistar Rats. Journal of Animal Research. 2020;10(2):215-220.
27. Kataria SK, Chhillar AK, Kumar A, Tomar M, Malik V. Cytogenetic and hematological alterations induced by acute oral exposure of imidacloprid in female mice. Drug Chem Toxicol. 2016;39(1):59-65. DOI: 10.3109/01480545.2015.1026972.
28. Tonietto BD, Laurentino AOM, Costa-Valle MT, Cestonaro LV, Antunes BP, Sates C *et al*. Imidacloprid-based commercial pesticide causes behavioral, biochemical, and hematological impairments in Wistar rats. Environmental Toxicology and Pharmacology. 2022;94:103924. DOI: 10.1016/j.etap.2022.103924.
29. 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. DOI: 10.4103/0971-6580.75843.
30. Benjamin MM. Outline of Veterinary Clinical Pathology. 3rd edition. Iowa State University Press; c1978.
31. John PJ. Alteration of certain blood parameters of freshwater teleost *Mystus vittatus* after chronic exposure to Metasystox and Sevin. Fish physiology and Biochemistry. 2007;33(1):15-20.
32. Kobir MA, Akther L, Hasan I, Shahid MAH, Haque Z, Karim MR. Effects of Imidacloprid-Contaminated Feed Exposure on Hematological Parameters in Adult Rabbits (*Oryctolagus cuniculus*). Research in Agriculture Livestock and Fisheries. 2020;7(3):439-444.
33. Kumar A, Tomar M, Kataria SK. Effect of sub-lethal doses of imidacloprid on histological and biochemical parameters in female albino mice. IOSR Journal Of Environmental Science, Toxicology And Food Technology. 2014;8(1):9-15.

34. Soujanya S, Lakshman M, Kumar AA, Reddy AG. Evaluation of the protective role of vitamin C in imidacloprid-induced hepatotoxicity in male Albino rats. Journal of Natural Science, Biology, and Medicine. 2013;4(1):63-67. DOI:10.4103/0976-9668.107262.
35. Vohra P, Khera KS, Sangha GK. Physiological, biochemical and histological alterations induced by administration of imidacloprid in female albino rats. Pesticide Biochemistry and Physiology. 2014;110:50-56. DOI:10.1016/j.pestbp.2014.02.007.
36. Sathiavelu J, Senapathy GJ, Devaraj R, Namasivayam N. Hepatoprotective effect of chrysin on prooxidant-antioxidant status during ethanol-induced toxicity in female albino rats. Journal of Pharmacy and Pharmacology. 2009;61(6):809-817. DOI:10.1211/jpp/61.06.0015.
37. Jadon A, Bhadauria M, Shukla S. Protective effect of *Terminalia belerica* Roxb. and gallic acid against carbon tetrachloride induced damage in albino rats. Journal of Ethnopharmacology. 2007;109(2):214-218. DOI:10.1016/j.jep.2006.07.033.
38. Badawy MH, Ahmed NS, Attia AM. Sub-acute oral toxicity of Imidacloprid and Fipronil pesticide mixture in male albino rats; biochemical and reproductive toxicity evaluation. Journal of Materials and Environmental Science. 2018;9: 2431-7.
39. Qadir S, Latif A, Ali M, Iqbal F. Effects of imidacloprid on the hematological and serum biochemical profile of *Labeo rohita*. Pakistan Journal of Zoology. 2014;46(4):1085-1090
40. Arfat Y, Mahmood N, Tahir MU, Rashid M, Anjum S, Zhao F *et al*. Effect of imidacloprid on hepatotoxicity and nephrotoxicity in male albino mice. Toxicology Reports. 2014;1:554-561. DOI: 10.1016/j.toxrep.2014.08.004.
41. Toor HK, Sangha GK, Khera KS. Imidacloprid induced histological and biochemical alterations in liver of female albino rats. Pesticide Biochemistry and Physiology. 2013;105(1):1-4. DOI: 10.1016/j.pestbp.2012.10.001.
42. Mahajan L, Verma PK, Raina R, Sood S. Toxic effects of imidacloprid combined with arsenic: Oxidative stress in rat liver. Toxicology and Industrial Health. 2018;34(10):726-735. DOI: 10.1177/0748233718778993.