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SUBLETHAL EFFECTS OF IMIDACLOPRID ON HAEMATOLOGICAL AND BIOCHEMICAL PROFILE OF FRESHWATER FISH, *CYPRINUS CARPIO*

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ABSTRACT: Imidacloprid is a commercialized Neonicotinoid pesticide, widely used to kill piercing and sucking types of insect pests in agriculture field. Due to its systemic nature and high water solubility, imidacloprid gradually reach and contaminate surrounding water bodies. The present study was planned to investigate the sublethal toxic impacts of pesticide imidacloprid on haematological and biochemical profile of freshwater fish, *Cyprinus carpio*. The fingerlings of *Cyprinus carpio* were exposed to three different sublethal concentrations of imidacloprid (7.8 ppm, 15.6 ppm and 23.4 ppm) for 30 days. Blood samples from all treatments were collected and used for hematological as well as biochemical diagnosis. During the trial period, haematological parameters like TEC, Hb and Hct were decreased significantly ($P<0.05$), whereas TLC count was found to be increased in imidacloprid treated fish. Similarly MCH count was lower but MCV was higher significantly as compared to control. In the biochemical analysis, plasma protein levels were significantly decreased in all the treatment groups when compared to control. Similar trends of significant decrease in the values of albumin and globulin was also reported. Thus, results of present study show that imidacloprid can cause remarkable alterations in the haematological and biochemical parameters of *Cyprinus carpio*.

KEYWORDS: *Cyprinus carpio*, imidacloprid, haematological, blood, protein.

INTRODUCTION

Pesticides and fertilizers are widely used in agriculture fields, fish farms and households to control the pest population including harmful microbes. These pesticides reach to nearby aquatic ecosystem either via run-off water, through soil or directly by spraying on water body[1] and adversely affects non-target aquatic animals such as fishes and prawn causing great economics loss.[2]

Imidacloprid is a commercialized Neonicotinoid pesticide, used to kill piercing-sucking types of insect pests in green houses and agriculture fields.[3] It is one of the best-selling insecticides, used as replacement for more toxic organophosphates and organochlorines.[4] Imidacloprid is water soluble crystalline solid pesticide whose molecular formula is $C_9H_{10}ClN_5O_2$ and molecular weight is 255.7.[5] It is a neurotoxic pesticide, binds with receptor for acetylcholine.[6] The systemic nature of imidacloprid assists it to bioaccumulate in plant tissues and protects the plant from early pest infection.[7] Its hydrolytic half-life is more than 30 days[3] and insecticidal activity perseveres for more than 90 days.[8] Along with agricultural runoff, imidacloprid reach and contaminate the surrounding water body creating toxicity for aquatic animals including fishes.[9]

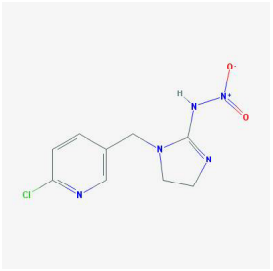
Aquatic toxicity of pesticides is generally difficult to assess but their monitoring is very important, as it makes ecological imbalance leading to loss of economic aquatic organisms.[10] Most of applied pesticides enter into fish body along with food or absorption through skin or gills and causes significant haematological and biochemical impairments.[11] Fishes usually have low blood volume (30-40 ml/kg) as compared to other vertebrates with cellular constituents of nucleated RBC and WBC.[12] Any change in haematological parameter represents an index of physiological disorder as it immediately responds to change in physico-chemical property of surrounding environment.[13] In present, haematological and biochemical parameters widely used in toxicological research for screening the physiological and pathological alterations and provide valued information about fish health under toxic stress of environmental pollutants.[14]

There is lack of data regarding the toxicity of Imidacloprid on haematological and biochemical profile of freshwater fish species present in India, therefore its toxicological profile needs to be explored thoroughly. Hence, the present work was planned to evaluate the acute and sublethal toxicity of Imidacloprid on some hematological and biochemical parameters of fish, *Cyprinus carpio* as it is an important candidate in aquaculture systems in India.

MATERIALS AND METHOD

Selection and maintenance of experimental fish: The healthy fingerlings of *Cyprinus carpio* having 10±1 gm body weight, procured from fish seed farm Mandheri (District Kurukshetra) were used as test organism. To avoid dermal infection, the fingerlings were bathed in 0.05% KMnO4 solution and acclimatized in plastic tubs of 50 L capacity for three weeks in the laboratory conditions at Department of Zoology, Kurukshetra University, Kurukshetra. During experimental period, water temperature was maintained at 27±1°C with normal lighting schedule of 12 hrs light and 12 hrs darkness. During acclimatization and experimental period fishes were fed with the diet containing 40% protein @ 4% BW day^{-1}.

Insecticide used: For the present study, pesticide imidacloprid (17.8 % SL) Manufactured by Gharda Chemicals Ltd. 48 Hill Road, Bandra, Mumbai, India and purchased from the local market of Kurukshetra, India, was used as toxicant.



Chemical structure of Imidacloprid (https://pubchem.ncbi.nlm.nih.gov/)

Determination of LC_{50}: LC_{50} or Median Lethal Dose (LD_{50}) is that concentration of test chemical which is supposed to kill 50 % population of test

organism. In the present study 48h LC_{50} for fertilized eggs of *Cyprinus carpio* was calculated by using semi-static method. The fertilized eggs were exposed to several concentrations of imidacloprid with initial dose of 5 ppm. The chemical dose was increased continuously to determine the concentration responsible for death of 50 % of the eggs. At each concentration, mortality was recorded and data was analyzed for probit analysis by software IBM SPSS Statics Version 20. The calculated LC_{50} was found to be 78 ppm for fertilized eggs.

Experimental Set-up: Three different sublethal concentrations of imidacloprid i.e. 7.8 ppm (10% of LC_{50}) coded as T_1, 15.6 ppm (20% of LC_{50}) coded as T_2 and 23.4 ppm (30% of LC_{50}) coded as T_3, were selected for further experimentation. These selected concentrations were used both for embryo toxicity test and fingerling toxicity test. Ten fingerlings (previously acclimatized) were exposed to each selected concentration along with control in triplicates for 30 days in plastic tubs of 50 L having 30 L dechlorinated water. To maintain hygienic condition, the tubs were cleaned everyday by siphoning. O_2 level was maintained by using low pressure aerators. The pesticide concentration was maintained by renewing the water in the tubs on alternative day's with water having respective concentration. Physico-chemical properties of control and treated water were analyzed according to the standard methods.

Collection of blood sample: Five fingerlings from each treatment group were selected for blood sample along with control in triplicates. Blood samples were collected from caudal vein and cardiac puncture, using plastic syringe (disposable) fitted with 26 gauge needle. Both syringe and needle were rinsed with anticoagulant heparin, manufactured by Biological E Ltd, Hyderabad, India. The collected blood samples were transferred to separate heparinized plastic vials and placed on ice immediately. The whole blood was used for estimation of RBC, WBC and Hb count. Remaining blood samples were centrifuged at 10,000 rpm for 20-25 min to separate blood plasma for estimation of plasma proteins.

Haematological Analysis

Total Erythrocyte Count (TEC): Total Erythrocyte Count (RBC) was calculated by using the haemocytometer (Neubaeur's counting chamber) method described by Dacie and Lewis.[15] Dacies fluid (Formaldehyde-10 ml, Brilliant cresyl blue-1.0 g, Trisodium citrate-31.3 g, Distilled water-1 ltr) was used diluting agent. The blood was drawn into RBC pipette upto 0.5 mark followed by immediate filling of diluting agent 101 mark. The pipette was shaken and initial few drops were discarded. Tip of pipette was permitted to touch the junction of Neubauer's slide and cover slip. RBC present within the boundaries of central small squares (80 smallest squares) were counted and TEC was calculated as per given formula.

TEC = total number of cells in five small squares x 10,000 mm^3 of blood.

Total Leukocyte Count (TLC): Total Leukocyte Count (WBC) was also calculated by haemocytometer (Neubaeur's counting chamber) method given by Dacie and Lewis.[15] Shaw's solutions A and B were used as diluting agents. Solution A:- Neutral red-25 mg, Sodium Chloride-0.9 g, Distilled water 100 ml. Solution B:- Crystal violet-12.0 mg, Sodium Citrate-3.8 g, Formaldehyde-0.4 ml, Distilled water-100 ml. Blood was drained in WBC pipette upto 0.5 mark followed by solution A up to half of blood and solution B up to 101 mark. WBC present within boundaries of four large squares were counted.

TLC = total number of cells in four small squares x 500 mm^3 of blood.

Haemoglobin determination (Hb): Haemoglobin (Hb) count was estimated by Sahli's[16] method. HCl (N/100) was used for haemolysis. Hb count was estimated in (%) or in g dl^{-1}. The graduated tube was filled with HCl up to mark 20 and mixed with blood taken in micropipette up to mark of 20 cm. Mixture was allow to rest for 10-15 min and then diluted with distilled water drop by drop till the solution colour precisely matched with standard glass tube.

Haematocrit value (Hct) or Packed Cell Volume (PCV): Haematocrit value (Hct) is the amount of packed RBCs, expressed as per cent of total blood volume. It is the RBC fraction of whole blood. Hct was calculated by microhematocrit method given by Nelson and Morris,[17] in which anticoagulated blood was centrifuged for 30 minutes at 300 rpm. The erythrocytes sediments at bottom and a red blood cell column appears, called as haematocrit value.

Mean corpuscular volume (MCV):- it is average volume of red blood cell, expressed in μm^{-3} and calculated according to standard formula.

$$\text{Mean corpuscular volume (MCV)} = \frac{\text{Haematocrit (\%) x 10}}{\text{RBC count in millions}}$$

Mean Corpuscular Haemoglobin (MCH): It is average Hb content of red blood cell, expressed in pictograms (pg) equivalent to 10^{-12} g. MCH was calculated by following standard formulas.

$$\text{Mean Corpuscular Haemoglobin (MCH)} = \frac{\text{Haemoglobin (g/dl) x 100}}{\text{RBC count in millions}}$$

Biochemical analysis

Estimation of total serum proteins: Total serum (plasma) proteins were counted by Lowry's[18] method of protein estimation. For this, 0.10 ml of blood plasma from each sample is mixed with 0.90 ml of double distilled water followed by 5 ml of Lowry's reagent. Mixture was shaked well and allowed to stand for 10 mints at room temperature. To this, 0.5 ml of Folin Ciocalteu reagent was added, mixed properly and again kept at room temperature for 15 mints. A bluish colour appears and intensity was recorded in a UV spectrophotometer at 640 nm. Blank and standard BSA (20 mg of BSA in 100 ml DDW stored at 4°C), were also run

simultaneously. Calculated protein content was expressed as µg/ml.

Estimation of Albumin content: Albumin content was estimated according to bromocresol method given by Gordon et al.[19] For this, 0.05 ml of plasma from each blood sample was mixed with 5.0 ml of Albumin reagent. A standard solution was also prepared by adding 0.05 ml of standard albumin in 5 ml albumin reagent. Blank was also maintained simultaneously.

These solutions were mixed carefully and kept at room temperature for 10 min. Green colour appeared and colour intensity was recorded at 640 nm. Albumin content was calculated as following formula:

$$\text{Serum Albumin (µg/ml)} = \frac{\text{Optical Density of Test}}{\text{Optical Density of standard}} \times 100$$

Estimation of Globulin content: Globulin content was calculated as per standard formulae:

Globulin = Total protein – Albumin

Statistical analysis: ANOVA (analysis of variance) followed by Duncan's multiple range tests were used to test significant differences among treatment groups. Statistical significance was settled at a probability of $P<0.05$. All statistics were performed using IBM SPSS Statics Version 20 for Windows 8.

RESULTS AND DISCUSSION

Probit analysis method indicated that 48 hrs LC_{50} of imidacloprid for fertilized eggs of *C. carpio* was 78 ppm. Alterations in hematological and biochemical profiles of fingerlings of *C. carpio* during sublethal imidacloprid exposure are given in Table 1 and Table 2. No mortality was reported in any of the experimental group throughout experimentation.

Table 1: Haematological parameters of *Cyprinus carpio* after sublethal exposer of imidacloprid for a period of 30 days

Haematological parameters	Imidacloprid Treatments			
	Control	T_1	T_2	T_3
TEC ($10^6 mm^{-3}$)	2.20 ± 0.009^{A}	2.08 ± 0.007^{B}	1.98 ± 0.023^{C}	1.86 ± 0.004^{D}
TLC (10u mm^{-3})	1.28 ± 0.003^{A}	1.35 ± 0.003^{B}	1.34 ± 0.003^{B}	1.50 ± 0.02^{C}
Hb (g/100ml)	7.50 ± 0.06^{A}	6.50 ± 0.06^{B}	5.70 ± 0.06^{C}	4.70 ± 0.06^{D}
Hct/PCV (%)	28.80 ± 0.15^{A}	28.33 ± 0.08^{A}	27.17 ± 0.20^{B}	25.97 ± 0.08^{C}
MCV (µm^3 $cell^{-1}$)	130.92 ± 1.25^{A}	135.95 ± 0.41^{BC}	136.89 ± 2.57^{BC}	139.50 ± 0.33^{BC}
MCH (pg)	34.24 ± 0.35^{A}	31.34 ± 0.21^{B}	28.89 ± 0.58^{C}	25.42 ± 0.30^{E}

All values are Mean ± S.E of mean. Means with different letters in the same row are significantly ($P<0.05$) different. (Data were analyzed by Duncan's Multiple Range test)

Effect of imidacloprid on haematological parameters

Total Erythrocyte Count (TEC): During the present investigation, significant ($P<0.05$) decline in total erythrocyte count (TEC) of treated fingerlings was reported as compared to control (Table 1). After 30 days of exposure, maximum TEC count was reported in control group i.e 2.2±0.009 (10^6 mm^{-3}), which was significantly decreased to 2.08±0.007, 1.98±0.023 and 1.86±0.004 (10^6 mm^{-3}) in control, T_1, T_2 and T_3 treatment groups respectively (Figure 1).

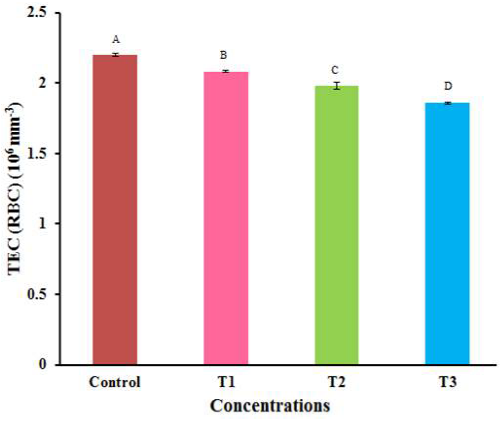


Figure 1. Total Erythrocyte Count (TEC) (10^6 mm^{-3}) of fingerlings of *C. carpio* exposed to sublethal concentrations of imidacloprid for 30 days. (Control= no pesticide, T_1= 7.8 ppm, T_2= 15.6 ppm, T_3= 23.4 ppm, T_4= 31.2 ppm). All the results are expressed as Mean ± S.E of mean, statistically significant ($P<0.05$) by one way ANOVA test; different letters indicate significant differences among means of different treatment groups after Duncan's Multiple Range test.

Total Leucocyte Count (TLC): Unlike TEC, a dose dependent significant ($P<0.05$) increase in TLC (WBC) count was observed from control to treatment T_3 (Figure 2). The raise in TLC is an indication of severe pathological condition due to imidacloprid toxicity. The reported TLC count in control group was 1.28±0.003 (10u mm^{-3}) which was increased to 1.35±0.003, 1.34±0.003 and 1.50±0.02 (10u mm^{-3}) in T_1, T_2, and T_3 treatment groups respectively (Table 1). However, the difference in the mean values of T_1 and T_2 group was found insignificant.

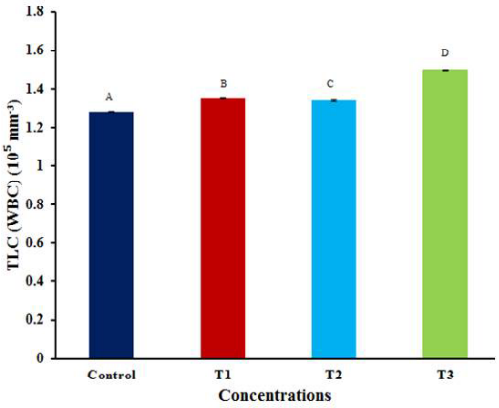


Figure 2. Total Leucocyte Count (TLC) (10u mm^{-3}) of *Cyprinus carpio* fingerlings exposed to sublethal concentrations of imidacloprid for 30 days. (Control= no pesticide, T_1= 7.8 ppm, T_2= 15.6 ppm, T_3= 23.4 ppm, T_4= 31.2 ppm). All the results are expressed as Mean ± S.E of mean, statistically significant ($P<0.05$) by one way ANOVA test; different letters indicate significant differences among means of different treatment groups after Duncan's Multiple Range test.

Haemoglobin determination (Hb): Like TEC Hb count of treated fingerlings also declined significantly ($P<0.05$) with increase in concentration of imidacloprid (Figure 3). Again maximum Hb count was recorded in control fingerlings i.e 7.5±0.06 (g/100ml), which was decreased to 6.5±0.06

in T_1, 5.7±0.06 in T_2 and 4.7±0.06 in T_3 (Table 1). The decline in Hb count is a result of decrease in TEC count.

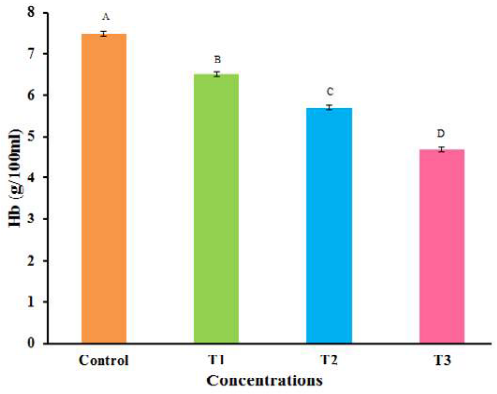


Figure 3. Haemoglobin (g/100ml) count of *Cyprinus carpio* fingerlings exposed to selected concentrations of imidacloprid for 30 days. (Control= no pesticide, T_1= 7.8 ppm, T_2= 15.6 ppm, T_3= 23.4 ppm, T_4= 31.2 ppm). All the results are expressed as Mean ± S.E of mean, statistically significant (P<0.05) by one way ANOVA test; different letters indicate significant differences among means of different treatment groups after Duncan's Multiple Range test.

Haematocrit Value (%) (Hct/ PCV): Hct values were decreased significantly (P<0.05) as compared to control showing a percent decrease from 28.80±0.15, 28.33±0.08, 27.17±0.20 and 25.97±0.08 in control, T_1, T_2 and T_3 treatment group respectively (Table 1, Figure 4).

Mean Corpuscular Volume (MCV): Concerning MCV, the count was increased significantly (P<0.05) with increase in pesticide concentration (Table 1). The increased MCV values with decrease in TEC and Hb count confirms haemolytic anemia and disruption in haemopoietic activities of fingerling exposed to pesticide.

Mean Corpuscular Haemoglobin (MCH): Mean corpuscular haemoglobin (MCH) again showed a significant (P<0.05) decline in dose dependent manner (Table 1). MCH has a maximum count of 34.24±0.35 in control group that declined significantly to 25.42±0.30 in T_3 treatment group.

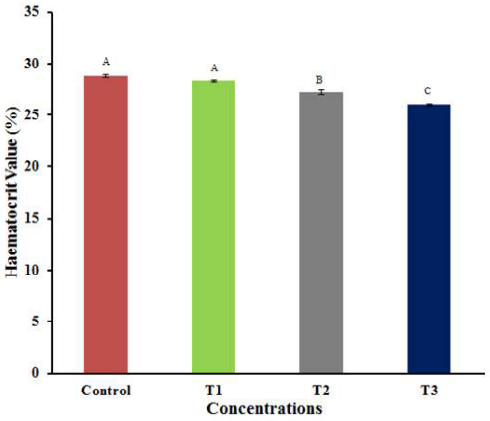


Figure 4. Haematocrit value (%) of fingerlings of *C. carpio* exposed to sublethal concentrations of imidacloprid for. (Control= no pesticide, T_1= 7.8 ppm, T_2= 15.6 ppm, T_3= 23.4 ppm, T_4= 31.2 ppm). All the results are expressed as Mean ± S.E of mean, statistically significant (P<0.05) by one way ANOVA test; different letters indicate significant differences among means of different treatment groups after Duncan's Multiple Range test.

Effect of imidacloprid on biochemical/ serological parameters

Variations in biochemical parameters like total protein, albumin and globulin levels are presented in Table 2. The results showed that prolonged pesticide exposure caused significant (P<0.05) decline in total serum protein count from 3.53±0.03 to 2.95±0.03 (μg mL^{-1}) in control and T_3

treatment group respectively (Figure 5). Similar trends of significant (P<0.05) decline in albumin and globulin (µg ml^{-1}) content was also reported from control to different treatment groups (Table 2). All the reported protein content were maximum in control group and decreased significantly (P<0.05) with increase in pesticide concentration (Figure 6).

Table 2: Biochemical (Serological) parameters of *Cyprinus carpio* exposed to sublethal concentrations of imidacloprid for a period of 30 days

Biochemical parameters	**Imidacloprid Treatments**			
	Control	**T_1**	**T_2**	**T_3**
Total protein (µg ml^{-1})	3.53 ± 0.03^{A}	3.49 ± 0.04^{A}	3.35 ± 0.03^{B}	2.95 ± 0.03^{C}
Albumin (µg ml^{-1})	1.85 ± 0.031^{A}	1.82 ± 0.028^{A}	1.74 ± 0.020^{B}	1.70 ± 0.01^{B}
Globulin (µg ml^{-1})	1.68 ± 0.015^{A}	1.66 ± 0.008^{A}	1.61 ± 0.015^{A}	1.25 ± 0.02^{B}

All values are Mean ± S.E of mean. Means with different letters in the same row are significantly (P<0.05) different. (Data were analyzed by Duncan's Multiple Range test)

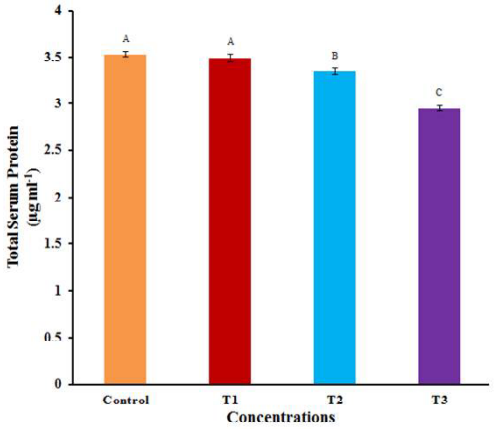


Figure 5. Total Serum Protein (µg ml^{-1}) count of *C. carpio* fingerlings exposed to selected concentrations of imidacloprid for 30 days. (Control= no pesticide, T_1= 7.8 ppm, T_2= 15.6 ppm, T_3= 23.4 ppm, T_4= 31.2 ppm). All the results are expressed as Mean ± S.E of mean, statistically significant (P<0.05) by one way ANOVA test; different letters indicate significant differences among means of different treatment groups after Duncan's Multiple Range test.

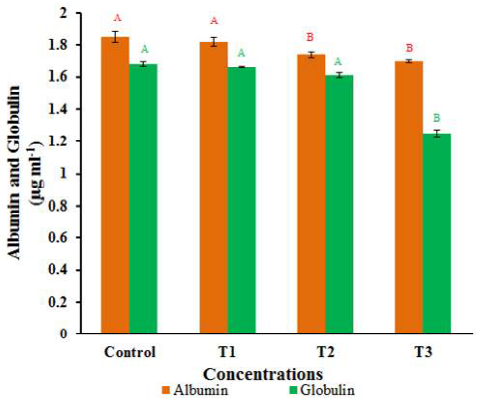


Figure 6. Albumin and Globulin (µg ml^{-1}) count of fingerlings of *C. carpio* exposed to sublethal concentrations of imidacloprid for 30 days. (Control= no pesticide, T_1= 7.8 ppm, T_2= 15.6 ppm, T_3= 23.4 ppm, T_4= 31.2 ppm). All the results are expressed as Mean ± S.E of mean, statistically significant (P<0.05) by one way ANOVA test; different letters indicate significant differences among means of different treatment groups after Duncan's Multiple Range test.

Imidacloprid is most commonly used neurotoxic insecticide, which binds and blocks the receptor for acetylcholine, required for proper neural transmission.[8] Over and repeated use of imidacloprid contaminates the aquatic environment and causes adverse effects on non-target organisms including fishes.[20] Fishes are most commonly used model organism in toxicological studies due to quick responding nature to toxicants even at low concentration.[21] The haematological parameters are ideal tools for screening of pathological stress and provide actual information concerning physiological conditions of fish.[22]

During present study, exposure of imidacloprid showed significant alterations in the haematological and biochemical parameters of *C. carpio*. There was a significant ($P<0.05$) decrease in haemoglobin (Hb) content, Total erythrocyte count (TEC), packed cell volume (PCV) and haematocrit (Hct) value with increase in pesticide concentration. However WBC count was increased significantly ($P<0.05$) in dose dependent manner. The decreasing trend of TEC with increasing concentrations of pesticide may be a result of decreased erythropoietic activity due to physiological stress caused by pesticides.[23] In vertebrates, including fishes, hormone erythropoietin produced by kidney is the key factor for maintaining erythropoietic activity as it regulates the production of coenzyme pyridoxal phosphate as well as pyruvate,[19] required in growing RBCs for hemoglobin (Hb) synthesis.[24] Thus, a decrease in TEC and Hb counts in imidacloprid treated fishes suggest the declined level of erythropoietin either due to severe dystrophic alterations in kidney tubules or declined activity of coenzyme pyridoxal phosphate.

Combs[25] also reported depletion in Hb as well as RBC count and concluded that insecticides might have adverse effect either on erythropoietin synthesis or coenzyme pyridoxal phosphate required for erythropoietic activity. Eisler and Edmunds[26] also reported similar decrease in haematological parameters in marine fishes after exposure of methyl parathion and methoxychlor. Declined RBC count and Hb content has been reported previously in *Clarias batrachus* after acute exposure of dichlorvos[27] and in *P. mesopotamicus* exposed of trichlorfon.[28]

Ahmadivand and coworkers[29] also reported significant decline in erythrocyte and Hb count, while a significant increase in neutrophils of male rainbow trout (*O. mykiss*) exposed to different doses of butachlor. The increased neutrophil count is a result of general immune response.[30] The decline in RBC and haemoglobin (Hb) count in pesticide exposed fish, might be a result of either decreased rate of synthesis or increase in the rate destruction.[31] According to Strmer and coworkers,[32] decline in Hb and RBC count is a result of toxicant induced conversion of haemoglobin to meta-hemoglobin. Another possibility of decreased Hb level is due to inflammation of RBCs and their poor acquisition of

haemoglobin during development in spleen.[33] Decline in Hb count fail to supply adequate oxygen and create a condition of oxygen deficiency (hypoxia) in toxicant exposed fishes.[34] Recently, Ismail and coworkers[35] recorded declined level of all hematological indices except TLC of freshwater fish, *Labeo rohita*, exposed to chlorpyrifos.

Further, variations in other haematological indices like significant decrease in MCH and increase in MCV values has also been recorded in the present investigation. These are very delicate parameters and required to maintain the homeostatic system of fish. Variations in any of these (MCH/MCV), might be a result of variation in RBC count, Hb and PCV count.

WBCs (Leukocytes) are important components of immunity and any changes in TLC counts indicate a declined functioning of immune system.[36] According to Siddique and Wanule,[37] increased leucocyte count may be a protective response against pesticide induced tissue damage or immunological response to detoxify the toxic substances present in different organs of exposed fish. Zubair[38] also reported increased TLC count in stressed fish and concluded that it is a characteristic response of leukocytes and is a result of leukocytosis such as lymphopenia and heterophilia.

Contradicting to our observation, progressive reduction in TLC count of fish, *P. Sophore* exposed to monocrotophos and endosulfan has been recorded by Dhembare and Pondha.[39] The decrease in WBC count under toxic stress might be a result of autolysis caused by leaked lysosomal enzymes of cell.[40] Kumar and Banerjee[41] also recorded similar observations of decreased leukocyte count in *C. batrachus* and concluded that it might be a result alteration in immunological set up of the stressed fish.

Along with haematological profile, toxicants also alter the structure, quality and quantity of serum proteins, therefore the serum proteins count is important biochemical parameter used to understand the broad status of fish health.[42] During present work, a significant ($P<0.05$) decline in total serum protein along with decline in albumin and globulin content was reported in treatment group when compared with control, which might be a result of severe hepatocellular damage under toxicant stress. Our observations were also supported by Singh and Singh,[43] who reported decreased plasma protein level and destruction as well as necrosis of cells in stressed *C. marulius*. Similar results have been also documented on different fishes viz., *Cyprinus carpio* exposed to dimethoate[44] and *O. mykiss* exposed to bifenthrin.[45] Another possibility of decreased protein level in pesticide exposed fish might be a result of directing the free amino acids towards the synthesis of necessary proteins or increased metabolic consumption of free amino acids in gluconeogenesis for extra energy production to manage toxicant stress.[46-47] Alterations in

carbohydrate metabolism and protein synthesis machinery also led to gradual decline in serum protein level.[48]

Protein albumin is a reserve protein that acts as osmoregulator and transporter protein, so any alteration in albumin level may cause severe pathological condition.[49] Globulin proteins are important components of immune system and provide resistance to fish against pathogens. The severe decline in albumin level in treated fishes suggests pesticide induce the renal disorder and liver insufficiency.[41] Patnaik[50] also concluded that decreased level of albumin and other proteins in stressed fish is a result of extra protein demand in tissue repair and detoxification mechanisms as impaired food intake is not able to supply sufficient energy. Prusty and coworkers[51] also concluded that most of animals including fishes mobilize their serum proteins as energy source under oxidative stress condition caused by pesticides. Velisek and coworkers[52] also described that declined protein level may be an indication of starvation, malnutrition, malabsorption liver inefficiency and renal disease in stressed fish.

CONCLUSION

From the present study, it is concluded that exposure of imidacloprid causes significant alterations in haematological and biochemical parameters of fingerlings of *C. carpio*. The findings also help to understand the toxicity mechanism of imidacloprid on fishes and can be to assess the safer level of this pesticide in the aquatic ecosystem for the protection of non-target aquatic organisms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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