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Acute Toxicity Impact of Herbicide Pretilachlor on Biochemical, Haematological and Ionic Changes in Gill, Liver and Kidney of a Cultivable Fish *Labeo rohita* (Hamilton).

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Abstract
Pretilachlor (PTC) is a systemic herbicide belonging to chloroacetamide group which is being used in rice fields for the control of weeds. The objective of the present study was to investigate the acute toxic effects of pretilachlor on a cultivable fish *Labeo rohita* (*L.rohita*) in gill, liver and kidney for 7, 14 and 21 days with 3 different concentration (1 mg/L, 3.9 mg/L and 5.9 mg/L). The animals were divided into four groups; control, Treatment I, Treatment II and Treatment III. *L.rohita* were exposed to PTC at a concentration of 1 mg/L (Treatment I), 3.9 mg/L (Treatment II) and 5.9 mg/L (Treatment III) for a period of 7, 14 and 21 days in gill, liver and kidney revealed numerous alterations were observed in PTC treated groups when compared to control group. The blood plasma glucose and protein levels were significantly ($P<0.05$) decreased throughout the treatment period when compared with control. The ionoregulation such as Na+, K+, and Cl- in gill and liver of *L.rohita* were significantly ($P<0.05$) decreased with increased ($P<0.05$) level of Cl- ion in liver tissue during throughout the treatment period when compared with control. However, a significant ($P<0.05$) increase level of Na+, K+, and Cl- in kidney tissue of *L.rohita* were observed throughout the treatment period when compared with control. In conclusion, the present study emphasizes that PTC at 1 mg/L (Treatment I), 3.9 mg/L (Treatment II) and 5.9 mg/L (Treatment III) for a period of 7, 14 and 21 altered the biochemical, ionic and hematological parameters of fish and these biomarkers serve as an effective test system for environmental risk assessment of aquatic environment.

Keywords
Pretilachlor; *Labeo rohita*; gill; Liver; Kidney; Toxicity

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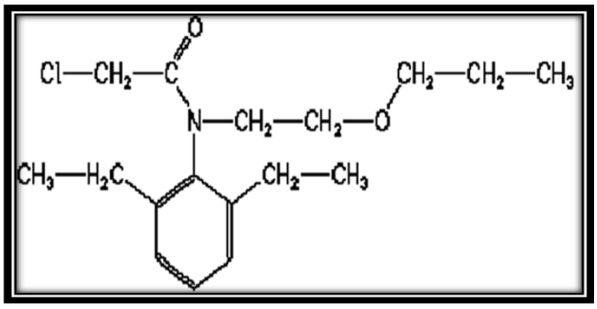
INTRODUCTION
In modern Agriculture, the usage of chemicals is increased for productivity of crops. Fertilizer used to increase the growth and pesticide used to protect crop against pest, due to increase in concentration of chemicals in environment, millions of cases the pesticide poisoning recorded in each year (Richter, et al., 2002). Pesticide include all substances which are

used to control insects, fungi and weeds and these substances are classified on the bases of organism which is target by pesticides, like insecticides, herbicides, fungicides, or fumigants (Kamel and Hoppin, 2004). Pests are one of the major yields limiting factors in rice productivity. Insects such as stem borer, leafhopper, plant hopper, gall midges are a group of defoliating species and a grain- sucking bug complex can causes significant yield losses.

Pesticides are classified into many groups, one among them the herbicides are widely used in the agriculture for a weed control. The majority of the herbicides target directly on the photosynthesis and indirectly through the inhibition photosynthetic pigment as well as the synthesis of other metabolites like lipids and proteins (Tomlin, 2000). Herbicides represent the largest proportion of pesticides used in agriculture. Worldwide use of pesticides in 2007 was estimated at 2.4 billion kg, of which largest proportion, 40% or 950 million kg, was herbicides (U.S. EPA, 2012a). The use of herbicides has increased the crop yield in agri-cultural production. However, with the increasing application of herbicides in modern agriculture, a large portion of these herbicides accumulate in surface water and pose environmental risks to aquatic organisms and human health. The chloroacetanilides are among the most widely used herbicides for pre-emergence control of undesirable grasses and broadleaf weeds in corn, cotton, soybean, and many other crops. The commonly used chloroactamide herbicides are acetochlor, butachlor, metolachlor, alachlor and pretilachlor. Evidences showed that most chloroactamide herbicides have been identified as probable carcinogens, acetochlor and alachlor can cause tumors in the nasal turbinates, butachlor causes stomach tumors, and metolachlor causes liver tumors (Coleman et al., 2000; Dearfield et al., 1999).

Fig. 1. Chemical structure of Pretilachlor



Pretilachlor (PTC) (the chemical structure is shown in Fig.1) is a selective pre-emergent herbicide widely applied to control annual grasses and broadleaf weeds in rice field. As a result of wide applications, it is one of the frequently detected pesticides in agriculture effluent and river water (Comoretto et al., 2007; Phong et al., 2010). Pretilachlor has a low toxicity to humans and mammals, however it is highly toxic to the aquatic organisms (Inderjit and Kaushik, 2010; Toan et al., 2013). Due to its chemical stability, pretilachlor is prone to accumulate in water bodies and is concentrated in aquatic organisms like fish and shellfish, bringing the potential risk to the aquatic ecosystems (Uno et al., 2001). The main sources of pretilachlor are manufacturing companies of herbicides and weedicides thus widely used in the agriculture industries. These residues reach into the aquatic environment by surface run-off causing risk hazards for aquatic flora and fauna. Among the aquatic organism, fishes were widely used to monitor water quality and also used as biological indicator of the polluted environment (Ramesh et al., 2018). *Labeo rohita*, a freshwater teleost fish belonging to the family Cyprinidae (Hamilton) is used for the present investigation. It is commonly called rohu. It is herbivorous, and it is one of the important major carps. This fish is abundantly available in all freshwater bodies. It is an edible fish, very rich in protein content and is available throughout the year. Few numbers of reports have been documented on the toxic effects of PTC on fishes (Rakesh et al., 2018). However, studies on the toxicity of PTC on Indian cultivable freshwater fish *Labeo rohita* are scanty. Hence, the present study is intended to explore the potential toxic effects of PTC at environmentally relevant concentrations on certain health biomarkers of edible fish *L.rohita* with special emphasis on biochemical, ionic regulation and hematological studies.

MATERIALS AND METHODS

Experimental animal collection and water quality parameters

The freshwater fish *Labio rohita* were collected from the Tamil Nadu Fisheries Development Corporation Limited, Aliyar Fish Farm, Aliyar, Tamil Nadu, and India. Fish were stocked in large cement tank (6'x 4'x 3') disinfected with potassium permanganate and washed thoroughly before the introduction of fish (to prevent fungal infection). After 15days acclimatization, fish with an average length of 7-8 cm and weighing 6-7 g were segregated and transferred to clean rectangular glass aquarium tanks (75 x 35 x 37 cm) of 100L water capacity. Media were changed to avoid fungal growth frequently and contamination by metabolites. These stocks were well aerated with aerators. During this time they were fed every 24 hour with a commercial diet. The physico-chemical parameters of the water were monitored throughout the acclimation period and remained constant (pH: 7.18 ± 0.5, conductivity: 118.25 ± 8.7 µS cm-1, dissolved oxygen: 8.49 ± 0.9 mg O_2 L-1, temperature: 21.96 ± 2.7 ^{0}C and total hardness 17.1 ± 0.8 mg/L were recorded throughout the study period. No mortality was found during the acclimation period. The water in the aquarium was renewed daily by removing three fourth of the water along with excess feed and faecal matters. Feeding was ceased 24 h before the initiation of the treatments.

Experimental design and chronic toxicity study

All the chemicals used in the experiment were analytical grade. The compound Pretilachlor (Chloro-N- acetamide) was purchased from Sri Sakthi Agro Chemical, Gobichettipalayam, and Erode. Oxidized glutathione (GSSG) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 1-chloro-2, 4-dinitrobenzene (CDNB), 5, 5-dithiobis-2-nitrobenzoic acid (DTNB), was purchased from SD fine chemicals limited, Mumbai, India. The rest of the chemicals and biochemicals utilized were obtained from local firms (India) and were of analytical grade. A stock solution of Pretilachlor [2-Chloro-N-(2-ethyl-6-ethylphenyl)-N-(2-ethoxy-1-methylethyl) acetamide] (1000 ml) was prepared by dissolving 1ml of Pretilachlor in 1ml of Acetone and this solution is made up to 1000 ml. From this stock solution, appropriate quantity was taken as desired concentration was selected for this study which was the 1/10th of LC_{50} value for 7 (1.0 ppm), 14 (3.9 ppm) and 21 (5.9 ppm) day respectively. The exposure concentrations were determined based on the information provided in the literature (Rakesh et al., 2018). Healthy fingerlings (200 Nos) from the stock were equally distributed (50 Nos) in four glass tanks (100 L capacity). Then 1 mg/L, 3.9 mg/L and 5.9 mg/L of PTC was added to the glass tank and identified as Treatment I, Treatment II, and III respectively. The remaining tank was maintained as control. Three replicates were maintained for each concentration and control group. The glass tanks were aerated and the concentration of PTC (1.0 mg/L, 3.9 mg/L and 5.9 mg/L) was renewed daily after performing the cleaning process. The study was conducted for 21 days. At the end of every 7, 14, and 21 day, fingerlings from each group (Control, Treatment I, II and III) were sacrificed for further analysis. During the experiment period (21 days) no mortality was observed.

Collection and preparation of samples

At the end of every stipulated period, fingerlings from control and PTC treated (Treatment I, II and III) groups (n =10), were segregated and the blood was collected by heart puncture using prechilled heparinised 30-gauge needled syringe (medical grade disposable). The collected blood samples were immediately transferred to heparinized Eppendorf vials (at ice cold condition). A portion of the whole blood was used for haematological analysis. The remaining blood samples were centrifuged in a cooling centrifuge at 10,000 rpm for 20 min, and the plasma was separated and stored at 4 ◦C. After blood was drawn, the organs such as gills, liver, and kidney were excised and stored at ice cold condition. 100 mg of each tissue was homogenized with 0.25 M sucrose solution. The homogenates were centrifuged for 20 min at 6,000 rpm at 4 ◦C and a clear supernatant was obtained. The collected plasma and supernatant were used for biochemical, ionic regulation (plasma glucose and protein) and other enzymological studies.

BIOCHEMICAL ANALYSIS

Estimation of plasma glucose

To estimate the level of plasma glucose, 5 mL of O-Toluidine reagent was mixed with 0.1 mL of sample. Simultaneously a blank (5 mL of O-Toluidine reagent and 0.1 mL of deionised water) and standard (5 mL of O-Toluidine reagent and 0.1 mL of glucose standard) were also prepared. Samples were kept in a water bath (hot) for 10 min, cooled under running tap water and the colour intensity was observed against blank using UV-spectrophotometer (630 nm). The results were expressed in mg/100 mL (Cooper et al., 1970).

Estimation of plasma protein

The protein level of the sample was determined following the method of Lowry et al.
(1951) using bovine serum albumin as standard. To 0.10 mL of sample, a volume of 0.90 mL of deionised water was added. Simultaneously, 1 mL of deionised

water in a glass tube was used as the blank. The contents were mixed with 5 mL of solution C (mixture of 50 mL of solution A (2 g of sodium carbonate dissolved in 100 ml of 0.1 N NaOH) and 1 mL of solution B (500 mg of copper sulfate dissolved in 100 mL of 1% sodium potassium tartarate solution)), and kept undisturbed at room temperature for 10 min. Later, 0.5 mL of Folin-phenol reagent was mixed with the content and kept for 15 min at room temperature. The optical density of the contents was determined using UV-spectrophotometer (720 nm) and expressed in µg/mL.

Estimation of inorganic ions in blood plasma and gill tissue

Na+ and K+ were estimated by the method of Maruna (1958) and Cl^- was estimated by the method of Young et al., (1975) and Tietz (1990) using standard kit procedures.

Haematological analysis

Haemoglobin (Hb) concentration (g/dl) was estimated by following the cyanmethemoglobin method of Drabkin et al., (1946). Hct was determined by following the microhematocrit method and expressed in %, as described in Nelson and Morris (1989). RBC and WBC counts were determined using Neubauer's haemocytometer, as described in Rusia and Sood (1992). Erythrocyte indices of fish viz., MCV, MCH, and MCHC were calculated based on standard formulas, as follows.

MCV (fL) = Hct (%)/RBC count in millions/mm^3 x 10

MCH (picograms) = Hb (g/dl)/RBC count in millions/mm^3 x 10

MCHC (g/dl) = Hb (g/dl)/Hct (%) x 100

Statistical analysis

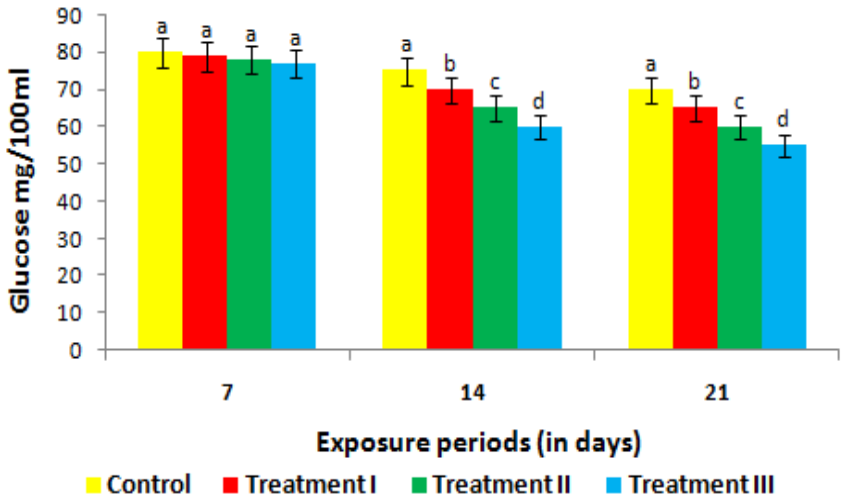
The results of the present study were expressed as means of five individuals of each parameter and the standard deviation was also measured. The significant differences between the control and treatments were compared using one-way ANOVA followed by Duncan's Multiple Range Test (DMRT). Result were considered significant at ($P<0.05$).

RESULTS

Effect of PTC on plasma glucose level

Maintaining adequate glucose levels in the blood or plasma are necessary for survival. On the other hand, inappropriate levels of glucose in the blood are a primary symptom of diabetes, a major degenerative disease in society. Fig. 2 shows the effect of Pretilachlor on plasma glucose level in control and experimental groups. There was a significant ($P<0.05$) decreased levels of plasma glucose in all three-treatment group I (7 days), II (14 days) and III (21 days) when compared with control group. No significant ($P<0.05$) changes were observed in the control group when compared with Pretilachlor treated groups.

Fig. 2. Effect of PTC on plasma glucose levels in control and experimental fish group.



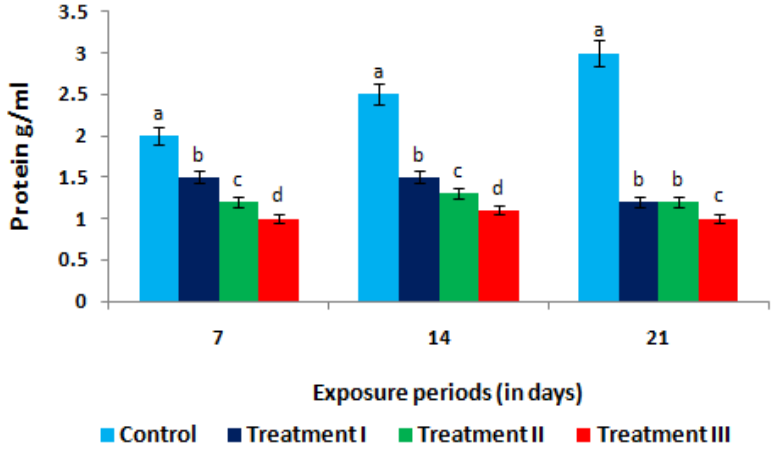
Values are expressed as mean ± SD of five individual observation. Statistical significance was determined by one-way ANOVA (Duncan's Multiple Range Test).

Effect of PTC on plasma protein level

Blood proteins, also termed plasma proteins are present in blood plasma. They serve many different functions, including transport of lipids, hormones, vitamins and minerals in activity and functioning of the immune system. In the present study, the level of plasma protein in Pretilachlor treated fish groups and control were shown in Fig. 3. The level of plasma protein was significantly ($P<0.05$) decreased in all the 3 different treated groups I (7 days), II (14 days) and

III (21 days) when compared with control group. No significant (P<0.05) changes were observed in the control group when compared with Pretilachlor treated groups.

Fig. 3. Effect of PTC on plasma protein levels in control and experimental fish group.

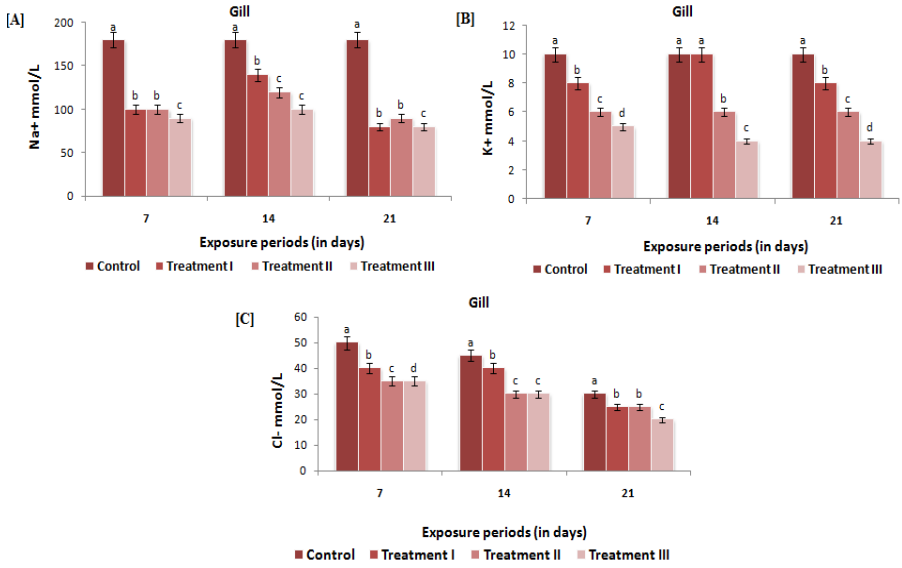


Values are expressed as mean ± SD of five individual observation. Statistical significance was determined by one-way ANOVA (Duncan's Multiple Range Test).

Effect of PTC on ion regulation (Na+, K+ and Cl-) in gill of *L.rohita*.

The effect of PTC on ionic changes (Na+, K+ and Cl-) in the control and experimental fish of *L.rohita* in gill were depicted in Figure 4. There was a significant (P<0.05) decrease level of Na+ (Fig.4A), K+ (Fig.4B) and Cl^- ion (Fig.4C) was observed in all the three-treatment group when compared with control. No significant (P<0.05) changes were observed in the control group when compared with Pretilachlor treated groups.

Fig. 4. Effect of PTC on ionic changes (Na+, K+ and Cl-) in gill of control and experimental fish group.

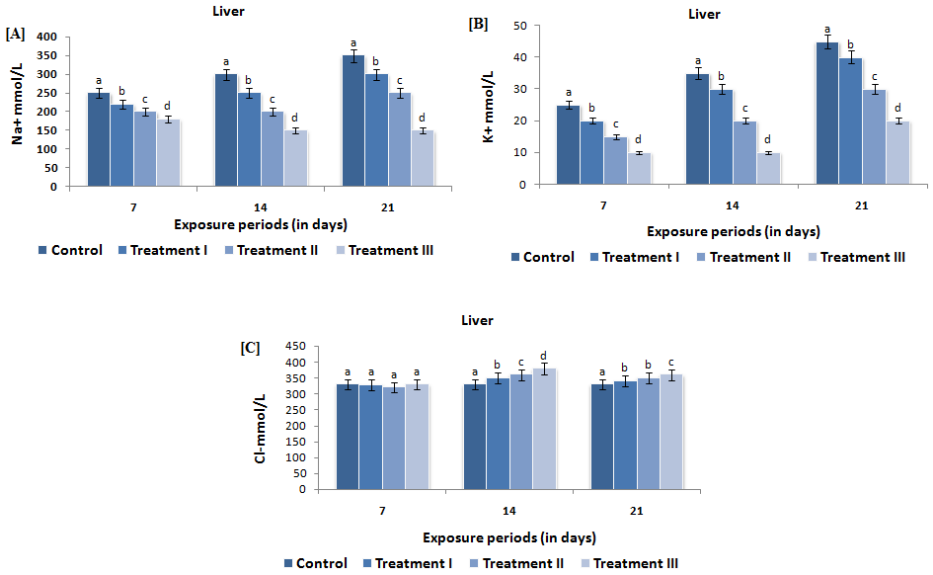


Values are expressed as mean ± SD of five individual observation. Statistical significance was determined by one-way ANOVA (Duncan's Multiple Range Test).

Effect of PTC on ionoregulation (Na+, K+ and Cl-) in liver tissue of *L.rohita*.

The effect of PTC on ionic changes (Na+, K+ and Cl-) in the control and experimental fish of *L.rohita* in liver tissue were depicted in figure 5. There was a significant (P<0.05) decrease level of Na+ (Fig.5A), K+ (Fig.5B) with increased level of Cl^- ion (Fig.5C) was observed in PTC treated groups when compared with control. No significant (P<0.05) changes were observed in the control group when compared with Pretilachlor treated groups.

Fig. 5. Effect of PTC on ionic changes (Na+, K+ and Cl-) in Liver of control and experimental fish group.

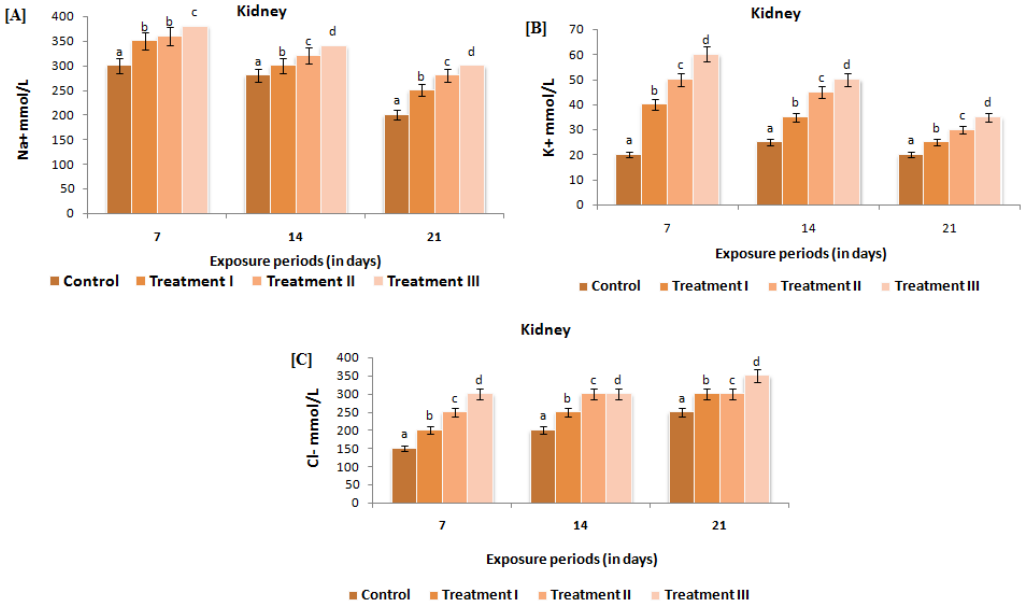


Values are expressed as mean ± SD of five individual observation. Statistical significance was determined by one-way ANOVA (Duncan's Multiple Range Test).

Effect of PTC on ionoregulation (Na+, K+ and Cl-) in kidney tissue of *L.rohita*.

The effect of PTC on ionic changes (Na+, K+ and Cl-) in the control and experimental fish of *L.rohita* in liver tissue were depicted in figure 6. There was a significant (P<0.05) increase level of Na+ (Fig.6A), K+ (Fig.6B) and Cl^- ion (Fig.6C) in all the three treatment groups when compared with control. No significant (P<0.05) changes were observed in the control group when compared with Pretilachlor treated groups.

Fig. 6. Effect of PTC on ionic changes (Na+, K+ and Cl-) in kidney of control and experimental fish group.



Values are expressed as mean ± SD of five individual observation. Statistical significance was determined by one-way ANOVA (Duncan's Multiple Range Test).

Effect of PTC on hematological parameters

The hematological profiles of L. rohita exposed to chronic concentration (Treatment I: 1 mg/L, Treatment II: 3.9 mg/L, Treatment III: 5.9 mg/L) of PTC and the control group were noted and the values were tabulated in Table 1. There was a significant (P < 0.05) decrease in the levels of Hb, Hct, and erythrocytes were observed in the blood of PTC

exposed fingerlings than the control group, for a period of 7, 14 and 21 days. Whereas, the values of leukocytes, and erythrocyte indices such as MCV, MCH, and MCHC were significantly ($P < 0.05$) increased in all PTC treated groups (I, II and III) throughout the exposure period when compared to the control group.

Table 1. Effect of PTC on haematological parameters

Parameter Exposure	Period in days	Control	Treatment I (1 mg/L)	Treatment II (3.9 mg/L)	Treatment III (5.9 mg/L)
RBC (million/cu.mm)	7	6.65 ± 0.005a	5.80 ± 0.005b	5.40 ± 0.012c	4.96 ± 0.098d
	14	6.67 ± 0.005a	5.60 ± 0.004b	5.50 ± 0.004c	5.20 ± 0.005d
	21	6.62 ± 0.007a	5.50 ± 0.004b	5.48 ± 0.007c	5.10 ± 0.014d
WBC (1000/cu. mm)	7	35.35±0.005a	35.37±0.005a	38.50±0.002b	39.48±0.003c
	14	33.36±0.002a	32.35±0.004b	31.38±0.005c	30.76±0.007c
	21	33.12±0.003a	33.10±0.001a	32.09±0.002b	30.18±0.006c
Haemoglobin (g/dl)	7	15.85±0.023a	13.38±0.024b	13.20±0.046c	13.10±0.040d
	14	13.76±0.013a	13.36±0.123b	13.15±0.051c	13.00±0.013d
	21	13.20±0.005a	13.18±0.004b	13.15±0.003c	13.05±0.002d
Hematocrit (%)	7	43.15±0.187a	43.15±0.195a	42.17±0.005b	42.05±0.014c
	14	40.82±0.022a	38.12±0.222b	38.12±0.066c	36.23±0.152d
	21	40.40±0.049a	40.30±0.321b	40.20±0.521c	39.19±0.813d
MCV (fL)	7	60.02±0.003a	61.97±0.142b	62.76±0.140c	63.56±0.023d
	14	62.38±0.305a	63.39±1.184b	63.18±0.172c	65.86±0.812d
	21	61.40±0.398a	65.40±0.183b	70.13±0.140c	70.18±0.175d
MCH (picograms)	7	20.80±0.045a	21.75±0.145a	22.30±0.065b	22.48±0.152c
	14	18.09±0.065a	19.64±0.074b	20.23±0.052c	21.32±0.326d
	21	18.32±0.012a	19.21±0.005b	19.18±0.001b	20.42±0.005c
MCHC (g/dl)	7	31.72±0.121a	32.60±0.132b	32.85±0.400b	33.02±0.012c
	14	32.52±0.171a	32.60±0.021b	33.62±0.291c	33.32±0.217c
	21	30.08±0.312a	31.10±0.003a	32.25±0.273b	33.34±0.218c

Values are means ± S.D of five individual observations, Different labels (a, b, c, d) of alphabets indicate significant differences at P < 0.05 between groups.

DISCUSSION

Biochemical parameters are widely studied to monitor health condition and biological functions of an organism, especially glucose and protein are routinely analyzed in the field of toxicology (Vutukuru 2003). In the present study described the toxicity of PTC on plasma glucose, protein and enzymatic antioxidants in gill, liver and kidney of fishes (*Labeo rohita*). The current study revealed that the PTC induced oxidative disturbance was occurred in the aquatic organism *Labeo rohita* gill, liver and kidney.

Determination of plasma glucose is important for the evaluation of islet function. Sufficient amount of glucose in the blood or plasma is necessary for survival. On the other hand, inapt levels of glucose in the blood are a primary symptom of diabetes. Glycolysis is one of the major pathways of ATP synthesis (Marvam et al., 2013). During the stress condition, fish need a high energy requirement, but the glucose level is fluctuated because of PTC induced toxicity. In the present investigation the level of glucose is decreased in Pretilachlor treated group at different time interval due to the hormonal imbalance in the *L. rohita* fish.

Proteins are important organic substances required by organisms in tissue building and play an important role in energy metabolism (Pang-Hung et al., 2008). Davinder et al., (2016) was observed the content of protein is decreased in the various concentrations of pretilachlor exposed to gill, liver and kidney tissues. A reduction in protein content was also observed in Cyprinus carpio and *Labeo rohita* exposed to cypermethrin substances (David et al., 2004). In the present study we observed that the reduction of protein content in the gill, liver and kidney of PTC treated group which was well corroborates with previous report of David et al., (2004). This may be due to the disturbance of energy metabolism and protein structure in the fish.

Ionic compounds balance is essential because freshwater fish are hyperosmotic to their environment. Freshwater fishes maintain their regular physiological process and body fluid homeostasis with the help of ion regulation process (Hwang et al., 2007). A gill is a respiratory organ

found in many aquatic organisms that extracts dissolved oxygen from water and excretes carbon dioxide. Gills of fish maintain pH of body fluid, the osmotic pressure of the body, nitrogenous wastes, and regulation of water influx and ion efflux. Ions like Na+, K+ and Cl- are important for the balancing the physiological activities of *L.rohita*. In the present study, there was a significant decreased level of Na+, K+ levels in gill, liver and increased Cl-ions liver in PTC treated groups when compared with control. Whereas, Na+, K+ and Cl-ion significantly increased when compared with control. This might be due to impairment of uptake mechanism of inorganic ion compounds at the gill (Sathya et al., 2012). Moreover, the toxic compound PTC may alter the membrane permeability attributing to a lesser intake of ionic compounds into the body. This could create intra-and extracellular fluid imbalance that resulted in hyponatremia, hypochloremia, and hypocalcemia in the fingerlings (Wood et al., 1996; Remen et al., 2008). Waterborne chemicals could act on the gill membrane and alters the permeability, which results in ion loss and decrease the plasma ion concentration (Uddin et al., 2016).

The hematological parameters are probably the more rapid and detectable variations under stress and are fuel in assessing different health conditions (Hymavathi et al., 2000). Hence, the haematological parameters in clinical and experimental studies in life sciences cannot be overemphasized. Particularly, literature reports have proved that the alterations in the haematological parameters, from normal state, may be used as valuable indicators of disease, or stress in different animal species (Miltonprabu and Thangapandiyan 2013). Changes in hematological, ionic compounds and biochemical parameters play a vital role in ecotoxicological evaluation of the chemicals in the environment (Lyon et al., 2010). Hb, Hct, RBCs, and WBCs as well as hematological indices such as MCV, MCH, and MCHC, are routinely used biomarkers with a wide potential for application in biomonitoring and aquatic toxicity studies (Miltonprabu and Thangapandiyan 2013). In the present study, decline in the Hb, Hct, RBCs, and WBCs content of the drug exposed group may be due to insufficient oxygen supply, and an indication of hypochromic microcytic anemia. Haemcontent has a significant role in energy metabolism, thus their changes could alter the mechanism of energy production.

Decline in the value of Hb content could also be due to the inhibitory effect of the drug on the enzyme system responsible for haemoglobin synthesis. Swelling of RBCs or the reduction in the rate of formation of RBCs could decline the Hct level in the organisms. The observed decrease in the RBC count indicates anemia, impaired osmoregualtion and gill damage (Poopal et al., 2017), caused by the PTC. Fish exposed to PTC showed leucocytosis, which is an indication of activation of immune system during abnormal conditions in organisms. This could result to protect them from PTC toxicity. The leucocytes from the spleen could also increase their count in the blood. The levels of erythrocyte indices such as MCV, MCHC and MCH in fingerlings were elevated; this could be due to the hypoxic condition created by the drug. The RBC swelling could contribute higher MCV in the circulation. MCH value could increase as a result of alterations in Hb, normally towards anaemic condition. Increased MCHC value indicates a protective response of the fish against the drug toxicity. This situation occurs due to congenital spherocytosis (haemolytic anemia), where the Hb concentration outside the cell is higher (Li et al., 2011). RakeshSoni et al. (2019) reported that the glucose phosphate isomerises (GPI) deficiency could lead to failure of the system that removes free radicals generated by PTC, thereby resulting in oxidation of haemoglobin and destabilization of red cell membranes, with acute hemolysis and severe hemoglobinuria.

CONCLUSION

On the basis of results obtained it can be concluded that the herbicide pretilachlor is toxic to fishes and its use should be in controlled manner to avoid its long-term harmful effects in the environment. The results of the present study indicate that PTC could cause significant changes in biochemical, ionic levels and hematological parameters of *Labeo rohita,* upon chronic exposure. The alterations of these parameters could be effectively used as potential biomarkers for the risk assessments of pharmaceuticals in the aquatic environments. The present study could provide baseline information on the potential effects of antibiotics on non-target organisms, especially on fish under chronic exposure. Further exploration of molecular toxicity studies would provide the better understanding of the amoxicillin action of antibiotics on organisms.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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