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Original Article

Effects of Chlorpyrifos on biochemical characteristics of Labeorohitafish during acute and chronic exposure

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ABSTRACT

Objective: The aim of present study was to examine the biochemical characteristics of Labeorohita fish during acute and chronic exposure of pesticide, chlorpyrifos (CPF). **Methods:** During acute exposure, fish were exposed to different concentrations of CPF ranging from 0, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.02, 0.03, 0.04 and 0.05 mg/L for 96 hrs in glass aquaria. The 96 hrs LC50 value of CPF for Labeorohita was found to be 0.01 mg/L. During chronic exposure fish were subjected to 1/3rd, 1/5th, 1/7th and 1/9th of LC50 for 30 days. At the end of the trial, samples were collected for biochemical (Blood cells, haemoglobin, plasma glucose and plasma protein) and sent to related laboratories. **Results:** The present study showed the RBCs, haemoglobin and Plasma Glucose level decreases as the concentration of CPF increases. On the other hand, platelets, WBCs and Plasma glucose level increases as the concentration of CPF increases. Decrease in RBCs count and haemoglobin indicates that CPF can cause anaemia to the fish. **Conclusions:** It is therefore concluded that Chlorpyrifos adversely affects the major organs of the fish Labeorohita.

INTRODUCTION

Pesticides are most destructive deadly chemicals introduced into the environment. Contamination by pesticides in aquatic ecological unit is a severe dilemma [1]. Generally the pesticides are genotoxic [2] and genotoxicity of pesticides is a worldwide concern [3]. Extensive use of pesticide which never anticipated the targets but create a large threat by entering the aquatic life. When exposed to sub-lethal concentrations of toxicants fish show severe physiological changes because fish are highly sensitive to water pollution [4]. For the enhancement of agricultural and industrial creation, waste water releases into freshwater, contaminates the water and causes toxicity in aquatic organisms when these pollutants enter the food chain. To evaluate the health of aquatic environment, fish are used and physiological changes in fish body indicating the changes in aquatic environment [5]. Aquatic environment is continuously being contaminated with toxic chemicals released from industrial, agricultural and domestic activities [6]. Sub-lethal concentrations of pesticides in aquatic environment is responsible for the disturbance of behavioral activities, rapid death and decrease the food consumption [7]. Pesticidal pollution responsible for the riskiest health hazard adversely effects on fish production. A range of contaminant sources affected the aquatic ecosystems [8]. Biological effects including biochemical alterations experienced on fish (non target organisms) are observed when exposed to pesticides [9]. Chlorpyrifos (CPF) is a chlorinated organophosphorus pesticide extensively

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used in agriculture and non-agricultural settings. CPF has lethal and sub-lethal levels of toxicities in aquatic environment. Lethal levels causes mass mortalities in fish and sub-lethal toxicities induce morphological, neurobehavioral, oxidative, biochemical, histopathological, haematological and developmental alterations [10]. CPF a crystalline organophosphate pesticide. It targets the nervous system of insects by inhibiting acetyl cholinesterase [11]. Several hundred per billion fish are died due the CPF according to a report [12]. CPF enters into water via air drift or surface runoff and stored by different aquatic organisms, particularly fish [13]. CPF can get in the way with steroid hormone production. As fish meat is important for human health and nourishment it getting value day by day in public and aquaculture [14] Dietary factors include omega-3 fatty acids and mineral contents. Nowadays, cultivation of fish is thought to be an excellent resource of vital fatty acids and is good for human use like the wild fish [15]. So, present study was designed to evaluate the effects of CPF under acute and chronic exposure on Labeorohita and its effects on blood and body of the fish.

METHODS

Experimental Fish: Healthy fingerlings of *Labeorohita* were purchased from Manawa Fish Hatchery, Lahore and brought to glass aquaria in the Fish Experiment Room, Animal House, Department of Zoology, Government College University, Lahore. Disinfected these specimens with table salt and potassium permanganate to prevent the fungal infection for 15 days prior to experimentation. The fish were fed with commercial food at least once a day during this period. Tap water was renewed every day with these physiochemical characteristics (Temperature 32 C and pH 7.3). The capacities of glass aquaria were 110 L.

Determination of LC_{50} (Median Lethal Concentration) Acute exposure of chlorpyrifos for *Labeorohita*: *Labeorohita* were starved 24hrs before beginning of the experiment. Four concentrations of CPF (0.008, 0.009, 0.01 and 0.02 mg/L) were prepared in four equal sized aquaria, in addition to one test aquarium for the control. Each aquaria contained 10 liter water and 15 fish individuals were transferred for each individual CPF containing aquaria. The fish were exposed to the prepared test solutions for 96 hours. The dead fish were watched and removed per day till the end of the fourth day. By the end of the fourth day, the mortality percentage was then calculated according to the profit analysis method. The experiment was repeated triplicate and the average of LC_{50} value for CPF was recorded as 0.01mg/L for 96hrs.

Chronic Exposure of Chlorpyrifos for *Labeorohita*: To determine the chronic toxicity fish were collected and randomly divided into five groups. The first group represents the control and other four groups were experimental groups. These groups labeled as T_1 (control group), T_2, T_3, T_4 and T_5 (experimental groups). LC_{50} value was recorded as 0.01mg/L. Each aquarium contains 40 Liters water and 20 fish individual were transferred. We took 1/3rd, 1/5th, 1/7th and 1/9th of LC_{50}. The fish were fed with commercial food at least once a day during this period. Daily removed the fecal matter from aquaria. This exposure was continued for 30 days. After 30 days samples were collected for different aspects and sent to the related laboratories.

Complete Blood Count (CBC) test: Test organism was removed, from each tank for blood analysis. From caudal peduncle 4- 5ml of blood was taken by using heparinized disposable syringes which contained 0.5mg of EDTA (ethylene diamine tetra acetic acid as an anticoagulant). After that blood was stored in deep freezer at -4°C prior to analysis.

Statistical analysis: The data were subject to one-way ANOVA, without any transformation, followed by least significant difference (Tuckey Test) test between the mean values to determine significant variation between the dietary levels. The data have been presented in the tables as mean ± SD.

RESULTS

Blood Parameters: Haematological changes in the selected haematological parameters of the control group and those exposed to CPF for the period of 30 days to *L.rohita*have been tabulated(Table 1) and plotted in Figure 1 (A-D) and Figure 2(A,B). Significant decrease was observed in RBCs, WBCs, Platelets, Hb, Plasma Glucose and PlasmaProtien exposed to CPF. In *L. rohita,* a significant elevated RBCs, WBCs, Platelets, Hb, Plasma Glucose and Plasma Protien values was observed in a dose dependent manner as compared to control.

RBCs: RBCs contents were recorded as 3.49±0.211, 2.52±0.345, 2.20±0.129, 1.89±0.115 and 1.53±0.104 in T_1, T_2, T_3, T_4 and T_5 experimental groups, respectively (Table 1 and Figure 1A). Maximum RBCs contents were observed in T_1 and minimum RBCs contents were observed in T_5, respectively. Analysis of Variance (ANOVA) showed that the differences were remained statistically significant ($P< 0.05$) for RBCs contents.

WBCs: WBCs contents were recorded as 8.57±0.473, 10.3±0.315, 14.1±0.540, 18.2±0.677 and 21.5±1.408 in T_1, T_2, T_3, T_4 and T_5 experimental groups, respectively (Table 1 and Figure 1B). Maximum WBCs were observed in T_1 and minimum WBCs contents were observed in T_5 experimental groups, respectively. Analysis of Variance (ANOVA) showed that the differences were remained statistically significant

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($P< 0.05$) for WBCs contents in T_3, T_4 and T_5 whereas remaining four groups T_1 and T_2 have non-significant with each other difference.

Platelets: Platelets contents were recorded as 41.2±0.789, 46.4±0.93, 53.6±1.876, 60.5±0.757 and 68.4±1.938 in T_1, T_2, T_3, T_4 and T_5 experimental groups, respectively (Table 1 and Figure 1C). Maximum platelets were observed in T_1 and minimum platelets were observed in T_5 experimental groups, respectively. Analysis of Variance (ANOVA) showed that the differences were remained statistically significant ($P< 0.05$) for platelet contents in all control and experimental groups.

Hb: Hb contents were recorded as 4.48±0.042, 4.36±0.030, 4.05±0.045, 3.74±0.276 and 3.73±0.160 in T_1, T_2, T_3, T_4 and T_5 experimental groups, respectively (Table 1 and Figure 1D). Maximum hemoglobin was observed in T_1 and minimum hemoglobin were observed in T_5 experimental groups, respectively. Analysis of Variance (ANOVA) showed that the differences were remained statistically significant ($P< 0.05$) for Hb contents in T_1 and T_2, whereas non-significant T_3 to T_5.

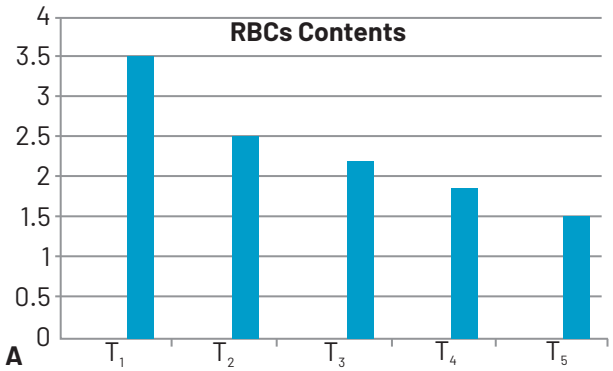
Plasma Glucose: Plasma glucose contents were recorded as 95.9±0.07, 110.8±0.036, 115.2±0.052, 120.9±0.040 and 123.7±0.108 in T_1, T_2, T_3, T_4 and T_5 experimental groups, respectively (Table 1 and Figure 2A). Maximum and minimum plasma glucose was observed in T_1 and in T_5 experimental groups, respectively. Analysis of Variance (ANOVA) showed that the differences were remained statistically significant ($P< 0.05$) for plasma glucose contents in all control and experimental groups.

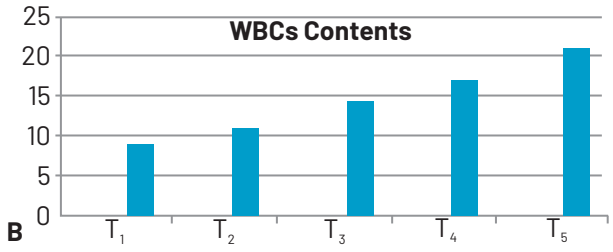
Plasma Protien: Plasma protein contents were recorded as 7.57±0.473, 6.87±0.045, 5.60±0.082, 4.76±0.07 and 3.93±0.020 in T_1, T_2, T_3, T_4 and T_5 experimental groups, respectively (Table 1 and Figure 2B). Maximum and minimum plasma protein was observed in T_1 and in T_5 experimental groups, respectively. Analysis of Variance (ANOVA) showed that the differences were remained statistically significant ($P< 0.05$) for plasma protein contents in all control and experimental groups.

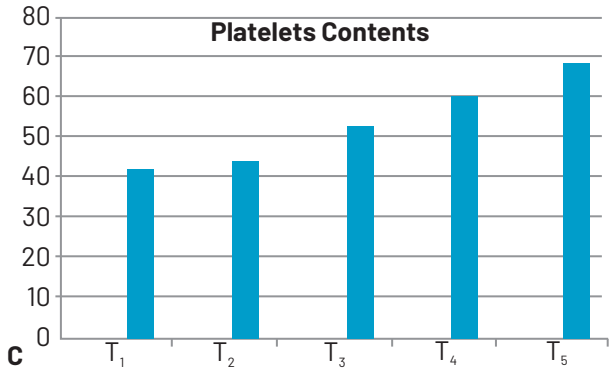
Treatment	RBC (Million/cu.mm)	WBC (Thousand/cu.mm)	Platelets (109 /L)	Hb (g/dL)	P. Glucose (mg/100 ml)	P. Protein (µg/ml)
T_1	3.49±0.211A	8.57±0.473D	41.2±0.789E	4.84±0.042A	95.9±0.07E	7.57±0.473A
T_2	2.52±0.345B	10.3±0.315D	46.4±0.93D	4.36±0.030B	110.8±0.036D	6.87±0.045B
T_3	2.20±0.129BC	14.1±0.540C	53.6±1.876C	4.05±0.045BC	115.2±0.052C	5.60±0.082C
T_4	1.89±0.115CD	18.2±0.677B	60.5±0.757B	3.74±0.276C	120.9±0.040B	4.76±0.07D
T_5	1.53±0.104D	21.5±1.408A	68.4±1.938A	3.73±0.160C	123.7±0.108A	3.93±0.020E

Table 1: Haemogram of L. rohitasubjected to LC50 of Chlorpyrifos

Means sharing similar letters in a column are statistically non-significant ($p>0.05$). T1 = control, T2 = 1/3rd of LC50 (0.008 mg/L), T3 = 1/5th of LC50 (0.009 mg/L), T4= 1/7thof LC50 (0.01mg/L) and T5= 1/9th of LC50 (0.02 mg/L).RBCs= Red Blood Cells, WBCs= White Blood Cells, Platelets, Hg= Hemoglobin, P. Glucose= Plasma Glucose, P. Protein=Plasma Protein







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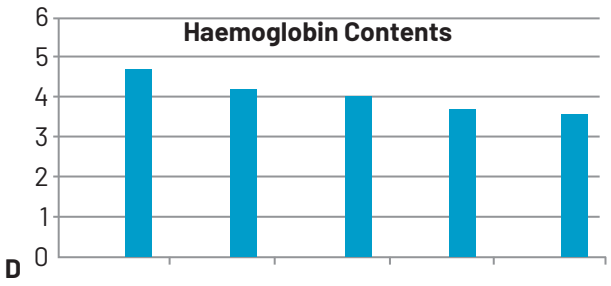

Figure 1A:Graph showing RBCs contents in different experimental groups with Chlorpyrifos, **1B:**Graph showing WBCs contents in different experimental groups with Chlorpyrifos, **1C:**Graph showing Platelet contents in different experimental groups with Chlorpyrifos, **1D:**Graph showing Hb contents in different experimental groups with Chlorpyrifos

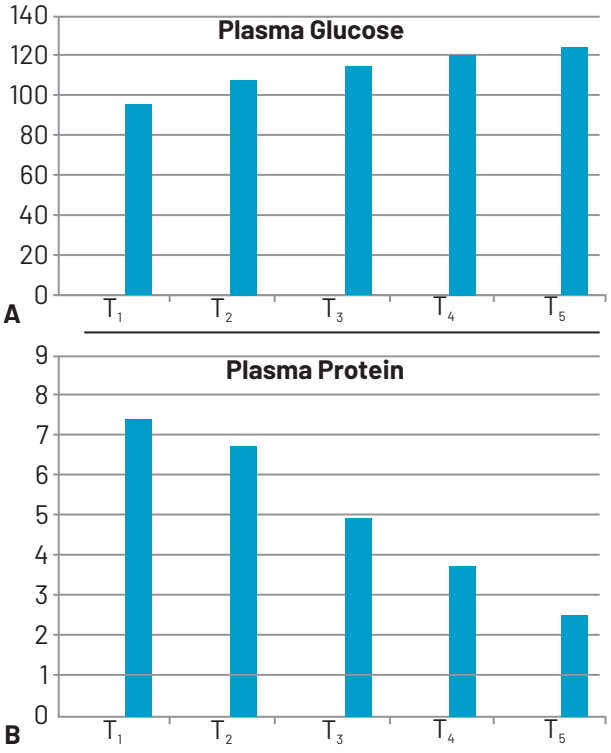

Figure 2A: Graph showing Plasma Glucose contents in different experimental groups with Chlorpyrifos, **2B:**Graph showing Plasma Protein contents in different experimental groups with Chlorpyrifos

DISCUSSION

The present study assessed the toxicity of a widely used organophosphate pesticide, chlorpyrifos with the evaluation of its effect on some blood parameters, histology and proximal composition of Labeorohita. The 96h LC50 values of CPF were determined for fingerlings of Labeorohita were found 0.01 mg/L or 10 µgL-1. The acute toxicity of CPF varied among different fish species and different age groups. The 96h LC50 of CPF for common carp were reported as 160µg/L [16] and 203µg/L [17]. Whereas, 96h LC50 of CPF for Oreochromismossambicus, Gambusiaaffinisand Channa punctatus were estimated as 154 µg/[18], 297µg/L, [19] and 0.811mg/L [20] respectively. The 96h LC50 value of chlorpyrifos-methyl for O. niloticuslarvae was 920 µg/L (Gul, 2005). Hematology is very important to assess the fish's health. The blood indicesvary with the variation of environmental conditions [21], reproductive activities and chemical stress [22]. The results of present study showed that RBCs count in T1 (control group)is 3.49%, T2(Experimental group)2.52%, in T3 2.20%, T4 1.89% and in T5 1.53%. The WBCs count in T1 8.75%, T2 13.3%, T3 14.1%, T4 18.2 and in T5 21.5%. In case of platelets T1 41.2%. T2 46.4%, T3 53.6%, T4 60.5% and T5 68.4%. In hemoglobin count T1 4.84%, T2 4.36%, T3 4.05%, T4 3.74% and T5 3.73%. In plasma Glucose T1 95.9%, T2 110.8%, T3 115.2%, T4 120.9% and T5 123.7%. In plasma protein T1 7.57%, T2 6.87%, T3 5.60%, T4 4.76% and T5 3.93%. The result showed the RBCs, Hemoglobin and Plasma Glucose level decreases as the concentration of CPF increases. On the other hand platelets, WBCs and Plasma glucose level increases as the concentration of CPF increases. Decrease in RBCs count and Hemoglobin indicates that CPF can cause anaemia to the fish. Decrease of RBC and hemoglobin may either be due to increased rate of erythrocyte destruction or inhibition of RBC formation and haemoglobin synthesis [23, 24]. Similar findings of decreased RBC and Hb have been reported when Labeorohitawas exposed to carbofuran and cypermethrin [25], and in C. carpioexposed to CPF [24]. The declined haemoglobin content as well as binding of CPF with haemoglobin rapidly reduced the amount of oxyhaemoglobin in blood and released of free reactive oxygen radicals [26]. Anaemia associated with erythropenia was reported for several freshwater fish species [27]. The decline of RBC and haemoglobin established a condition of erythropenia and stress that caused hemolysis. Lymphopoiesis stimulation and elevated synthesis of lymphocyte tissue under the effect of insecticide stress might be the reason of increased number of WBC production [28], as observed in 4 tetra-octylphenol exposed C. dimerus [29]. Similarly, the significant increase of WBC in CPF exposed fish L. rohitain our study might be due to the protective response against chemicals as reported by [30]. The increase in number of leucocytes is a defensive reaction against pesticide stress. These variations are probably due to immune system activation which may be an adaptive response in fish immune defense, as we observed in the present study [31, 32].

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