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Efficacy of Herbicide Glyphosate Hijack® on the Blood Parameters of the Freshwater Fish, *Catla catla* (HAM)

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Authors' contributions

This work was carried out in collaboration among all authors. Author FJF designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors Saradhamani and Mahalakshmi managed and guidance the analyses of the study. Authors FCMP managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out to study of the haematology of the freshwater fish, *Catla catla* were exposed to herbicide glyphosate hijack® for 10, 20 and 30 days in laboratory condition. Several haematological changes such as RBC, WBC, HB, HCT, Blood Glucose, Total Cholesterol, Triglycerides, Total Protein were observed in fish blood would serve as a useful purpose in evaluating the toxic effects of herbicide glyphosate hijack® (41%). All the values were significantly decreased from control. The maximum decreased level of activities in all these blood values was at 30 days. It has been concluded that herbicide glyphosate hijack ® (41%) at sub-lethal concentration may alter the RBC, WBC, HB, HCT, total glucose, total cholesterol, triglycerides and total protein in blood in all the exposure periods.

Keywords: Herbicides; RBC; WBC; HB; HCT and blood biochemistry.

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1. INTRODUCTION

Aquatic ecosystems can be contaminated by industrial and municipal discharges as well as surface runoff, which may cause alterations into the natural environment. Many chemical constituents of sewage including pesticides, herbicides, polychlorinated biphenyls (PCBs), heavy metals, petroleum oil, industrial oil, garbage leachate, detergents, cosmetic products, and pharmaceuticals could directly impact aquatic organisms by many researchers [1-3].

Fish haematology is known to be an essential tool to the fisheries biologist, as it acts as a frontline sensitive indicator of vital physiological and biochemical functions as well as the status of nutrition, health, diseases and stress responses of the organism subjected to changes in environmental conditions. Therefore, the striking alterations in the blood parameters and associated pathological changes in fishes under influence of various toxic agents have attracted the attention of workers in the field by Johnson et al. [4]. Blood is the medium of intercellular and intracellular transport, which comes in direct contact with various organs and tissues, the physiological state of an animal at a particular time is reflected in its blood by Rifai et al. [5]. Recently, much literature has been generated about the pollution effects of various chemicals on different organs and haematology of different fishes by many researchers [6-10].

2. MATERIALS

Catla catla, a commonly occurring freshwater major carp available in the local ponds and rivers were selected for experimental studies. A bulk of fish samples (*Catla catla*) ranging from 3-4 gm in weight and measuring 3-5 cm in length were procured from Bhavanisagar reservoir. Batches of 10 healthy fish *Catla catla* were exposed to different concentrations of herbicide glyphosate hijack® (41%) to calculate the LC_{50} value. It was found as 4.2 ppm for 96 hrs using probit analysis method by Finney, 1971 [11]. Three groups of fish were exposed to 0.42 ppm ($1/10^{th}$ of 72hrs LC_{50} value) concentration of the Herbicide Glyphosate Hijack® (41%) for ten days, 20 days and 30 days respectively. Another group was maintained as a control. At the end of each exposure period, blood was collected using a heparinised syringe from the gills, and various haematological parameters were analysed by using standard methods, RBC and WBC by Blaxhall and daisley [12], HB by Lee et al. [13], HCT by wintrobe [14]. The blood was immediately centrifuged at 1500rpm for 10 min. Serum then removed and stored at 40°C before immediate determination of biochemical parameters such as Plasma glucose concentrations were measured by colour-imetrically according to Trinder [15], total serum cholesterol (CHOD-PAP method by Naito et al. [16], serum triglycerides by Buccolo et al. [17], total protein was measured according to the method of Lowery et al. [18].

Statistical analyses of all data obtained from experimental groups were analyzed by Statistical Package for the Social Sciences (SPSS) 16.0 package program with Duncan's Multiple Range Test at significant values of $p< 0.05$. The standard deviation was also estimated.

3. RESULTS AND DISCUSSION

In the present investigation, the effect of herbicide glyphosate hijack® on biochemical nature of blood glucose, total cholesterol, triglycerides, total protein, albumin and globulin in the fresh water fish, *Catla catla* have been studied and presented as figures and tables.

There is a significant decrease in RBC observed in the long-term duration 2.30, 2.10 and 1.5 $10^6 \times mm^3$ in exposure and 2.60, 2.50 and 2.40 $10^6 \times mm^3$ in control respectively. The decrease in RBC count indicates acute anaemia. The anaemia could be due to the destruction of RBC or may be haemolysis of erythrocytes. Similar results were reported by Bhagat and Banerjee [19] the total erythrocytes counts are positively correlated with body length. Seasonal changes in RBC count and haemoglobin content were observed in a freshwater exotic fish reported by Yasmin et al. [20]. There was decrease level in RBC, HB and increase in WBC when the fish *C. punctatus* treated with malathion by Holmes and Donaldson [21]. Similarly, variations observed in blood cells count of *C. punctatus* due to toxicants reported by Menon [22].

The WBC in the blood of fish after exposed to different (10, 20 and 30) days in the 0.42 ppm concentration of glyphosate hijack® (41%) were found to contain 30.10, 32.20 and 26.70 $10^3 \times mm^3$ and in control, it contained 21.30, 22.10 and 22.90 $10^3 \times mm^3$ (Table 1 and Fig. 2). Increased leucocyte count in the blood of European chub establishes leucocytosis, which is considered to be of adaptive value for the tissue under pollution-induced chronic stress. This also helps in the removal of cellular debris

of necrosed tissue. In the presence of foreign substances or under pathological conditions leukocytosis in fish can be the consequence of direct stimulation of immunological defence stated by John [23].

The environmental changes and the pollutants in the water may increase the RBC/WBC ratio and also alter the physiological and chemical properties of fish blood parameters were studied by Yanik and Atamanalp [24]. According to [25] increased total leukocyte cell count in mono-sex Nile tilapia, *Oreochromis niloticus* exposed to an acute concentration of deltamethrin may be due to stimulated lymphopoiesis and enhanced release of lymphocytes from a lymphomyeloid tissue.

The total amount of HB in fish blood after exposed to different (10, 20 and 30) days in 0.42ppm concentration of glyphosate hijack® (41%) were found to contain 6.90, 6.30 and 4.50 gm% and in control it contained 7.80, 7.50 and 7.20 gm% (Table 1 and Fig. 3). The level of HCT in the blood of fish after exposed to different days were found to contain 20.70, 18.90 and 13.50% and control were found to contain 23.40, 22.50 and 21.60% (Table 1 and Fig. 4).

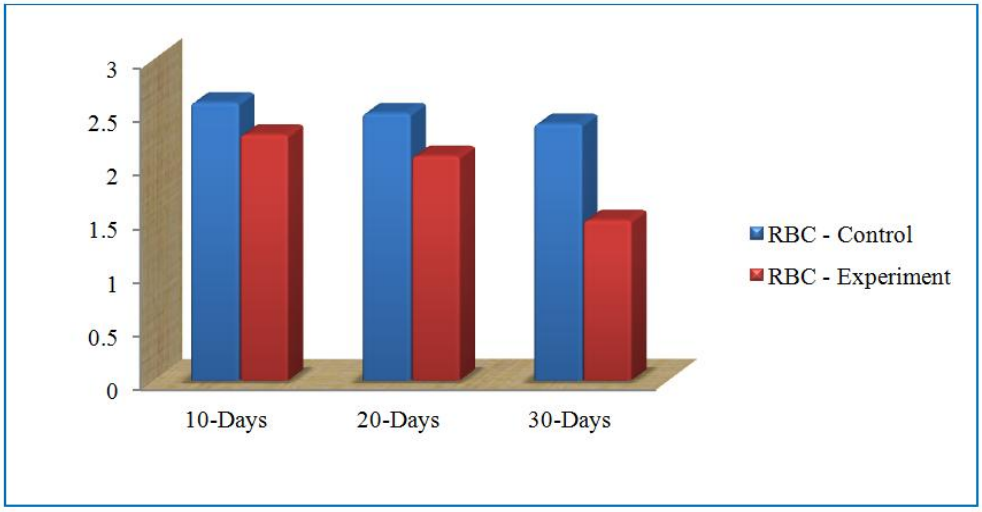

Fig. 1. Showing the changes in the blood of *Catla catla* exposed to 0.42 ppm of Herbicide Glyphosate Hijack® (41%) in long-term duration

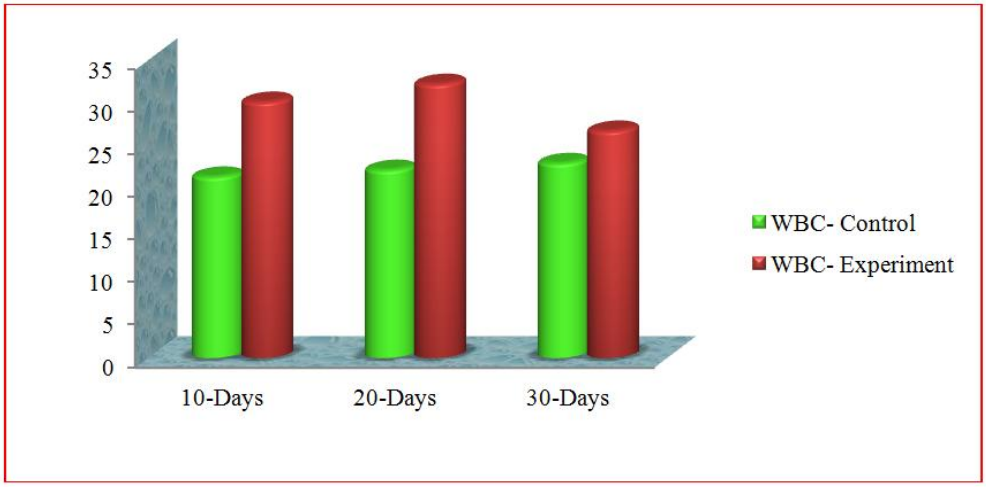

Fig. 2. Showing the changes in the blood of *Catla catla* exposed to 0.42 ppm of Herbicide Glyphosate Hijack® (41%) in long-term duration

Table 1. Showing the changes in the blood of *Catla catla* exposed to 0.42 ppm of Herbicide Glyphosate Hijack® (41%) in long-term duration

Duration	RBC (10^6×mm^3)			WBC (10^3×mm^3)			HB (gm %)			HCT (%)		
	C	E	MD	C	E	MD	C	E	MD	C	E	MD
10-Days	2.6 ±0.169^{a}	2.3 ±0.158^{ab}	0.3*	21.3±0.69^{a}	30.1±0.158^{ab}	-8.8***	7.80±0.29^{a}	6.90±0.158^{ab}	0.9***	23.4±0.77^{a}	20.7±0.158^{ab}	2.7***
20-Days	2.5 ±0.169^{a}	2.1 ±0.158^{ac}	0.4***	22.1±0.69^{a}	32.2±0.158^{ac}	-10.1*	7.50±0.29^{a}	6.30±0.158^{ac}	1.2***	22.5±0.77^{a}	18.9±0.158^{ac}	3.6***
30-Days	2.4 ±0.169^{a}	1.5 ± 0.158^{ad}	0.9***	22.9±0.69^{a}	26.7± 0.158^{ad}	-3.8***	7.2±0.29^{a}	4.50± 0.158^{ad}	2.7***	21.6±0.77^{a}	13.5± 0.158^{ae}	8.1***

Table 2. Showing the changes in the blood of *Catla catla* exposed to 0.42 ppm of Herbicide Glyphosate Hijack® (41%) in long-term duration

Duration	Blood glucose (mg/dL)			Total cholesterol (mg/dL)			Triglycerides (mg/dL)			Total protein (g/dL)		
	C	E	MD	C	E	MD	C	E	MD	C	E	MD
10-Days	71.6±2.28^{a}	73.2±0.15^{ab}	-1.6^{ns}	127±2.96^{a}	119±0.15^{ab}	8.0**	25.4±0.53^{a}	23.8±0.15^{ab}	1.6**	1.61±0.03^{a}	1.35±0.01^{ab}	0.16***
20-Days	74.5±2.28^{a}	82.3±0.68^{ac}	-7.8***	123±2.96^{a}	103±0.15^{ac}	20.0***	24.6±0.53^{a}	20.6±0.01^{ac}	4.0***	1.57±0.03^{a}	1.43±0.01^{ac}	0.14***
30-Days	69.1±2.28^{a}	85.2± 0.15^{ae}	-16.1*	121±2.96^{a}	97.6± 0.15^{ad}	23.4***	24.2±0.53^{a}	19.5± 0.01^{ae}	4.7***	1.54±0.03^{a}	1.27± 0.01^{ad}	0.30***

- ✓ *Values are Mean ± SD of five observations*
- ✓ *Duncan's multiple range test (DMRT) was used to evaluate the comparison between means as indicated by different case letters in a descending order a, b, c, d and e. (Significance was expressed using F-value at P<0.05.)*
- ✓ *Statistical significant : * - P < 0.05 , ** - P < 0.01 , *** - P < 0.001 , ns - Not significant*
- ✓ *Unit: C- Control / E- Experiment / MD – Mean Difference*

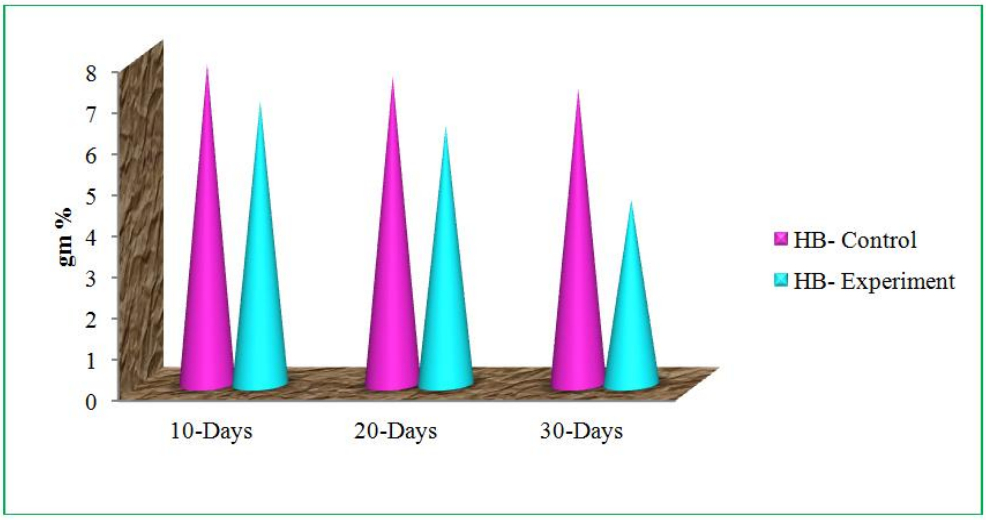

Fig. 3. Showing the changes in the blood of *Catla catla* exposed to 0.42 ppm of Herbicide Glyphosate Hijack® (41%) in long-term duration

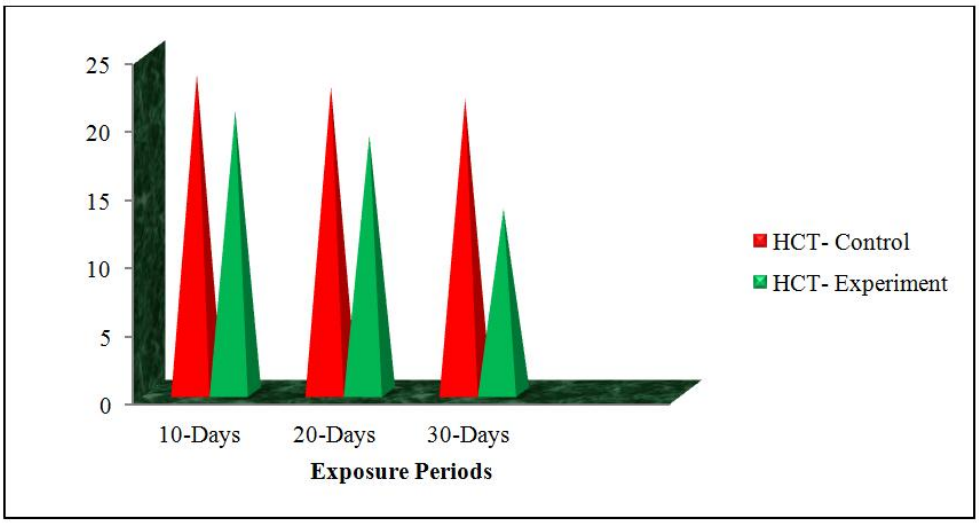

Fig. 4. Showing the changes in the blood of *Catla catla* exposed to 0.42 ppm of Herbicide Glyphosate Hijack® (41%) in long-term duration

Our findings were agreement with Zaki et al. [26] who reported that *Tilapia nilotica* infected with *Saprolegnia parasitica* resulted in a significant decrease in the total erythrocyte count, Hb and PCV. Similar findings were also reported by Hatai et al. [27] on naturally infected Ayu (*Plecoglosus altivelis*) with fungus *Aphanomysis piscicida*. Similar findings were also reported by Aldrin et al. [28] and Zaki et al. [26]. The lowest levels were recorded in *L. rohita.* Low Hct or PCV in the fishes probably indicated anaemia or hemodilution. A minimum level of Hct was noted that the various size according to Satheeskumar et al. [29] and Fazio et al. [30].

The random blood glucose level in 10, 20 and 30 days exposure were found to contain 73.20, 82.30 and 85.20 mg/dL and in control, it was found to be containing 71.60, 74.50 and 69.10 mg/dL (Table 2 and Fig. 5). The significant ($P < 0.05$) increase in glucose level may be considered to be manifestations of stress induced by the pesticides. Glucose increase is a general response of fish to acute and sub-lethal pollutant effects were reported by [31]. Increase in serum glucose levels in fish under stress was reported by many researchers [32-34]. This can be attributed to several factors, and one of them is

the decrease in the specific activity of some enzymes like phosphofructokinase, lactate dehydrogenase and citrate kinase that decrease the capacity of glycolysis. Similar studies were carried out by Saha and Kaviraj [35] in *Heteropneustes fossilis,* was reported by Banaee et al. [36] in *C. carpio* and Banaee et al. [37] in *O. mykiss* after exposed to cypermethrin and diazinon respectively.

In stressful situations, glucose is converted to pyruvate in the glycolytic pathway, and pyruvate is metabolized to acetyl-CoA in aerobic tissues, which can be used as a precursor in the synthesis of cholesterol and fatty acids in the citric acid cycle was reported by Murray et al. [38].

The level of cholesterol in the serum of fish after exposed to different (10, 20 and 30) days in 0.42 ppm concentration of glyphosate hijack® (41%) were found to contain 119, 103 and 97.60 mg/dL and in control it contained 127, 123 and 121 mg/dL (Table 2 and Fig. 6). Cholesterol is the base material for all steroid hormones. When it increases due to cortisol synthesis, then a large amount of cholesterol is needed was reported by [39]. Therefore, the reduction in the amount of cholesterol may be related to its utilization in the manufacture of Cortisol arising from stress created by the toxin herbicides.

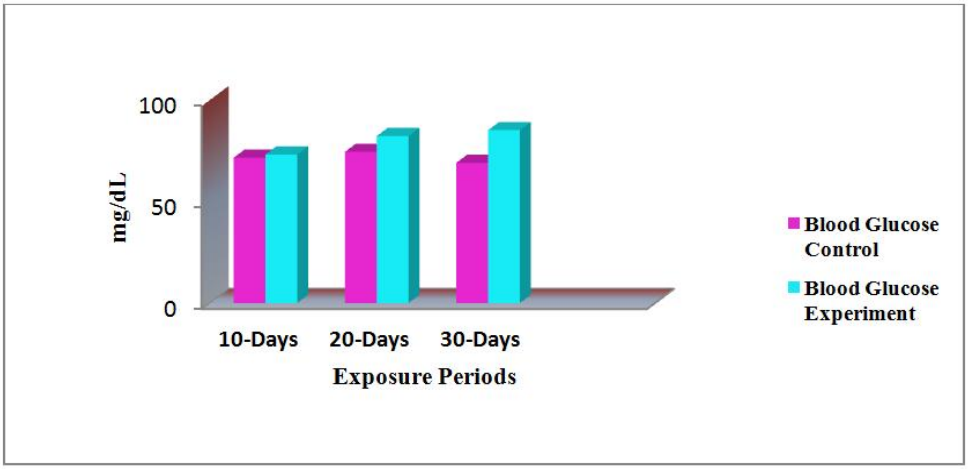

Fig. 5. Showing the changes in the blood of *Catla catla* exposed to 0.42 ppm of Herbicide Glyphosate Hijack® (41%) in long-term duration

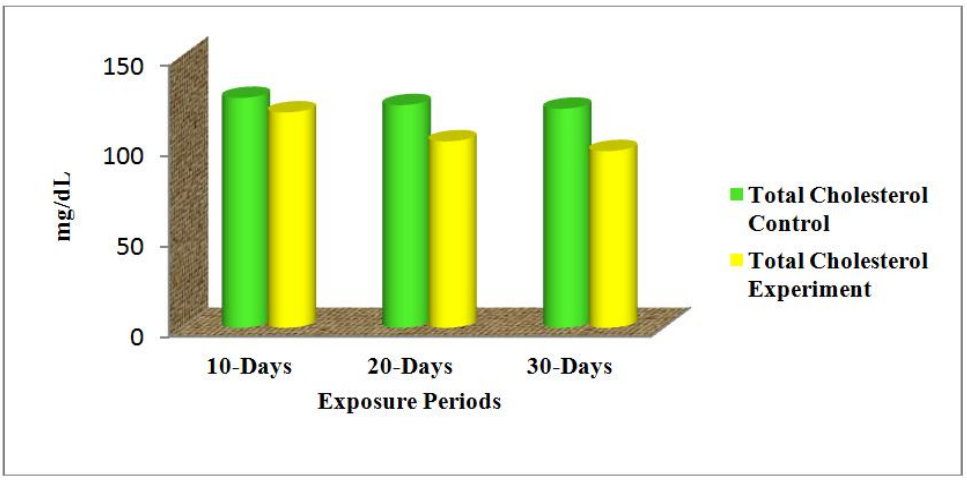

Fig. 6. Showing the changes in the blood of *Catla catla* exposed to 0.42 ppm of Herbicide Glyphosate Hijack® (41%) in long-term duration

The level of triglycerides in the serum of fish after exposed to different days were found to contain 23.80, 20.6 and 19.50 mg/dL and in control, it contained 25.40, 24.60 and 24.20 mg/dL (Table 2 and Fig. 7). Triglyceride is the storage form of fats and major resources of oils and fat which are flowing into the blood. The reduction of triglyceride volumes in blood plasma at high concentrations of the toxin glyphosate hijack® could be due to the imbalance created by the higher concentrations of the toxin, affecting the digestive system, liver and related enzymes as well as hormonal and natural metabolic imbalance in fish studied by Robinson [40].

There is a significant decrease in serum protein observed in the long-term duration 1.35, 1.43 and 1.27 g/dL in exposure and 1.61, 1.57 and 1.54 g/dL in control respectively (Table 2 and Fig. 8). This may be due to the toxic stress which may reduce protein content in tissues. Decreased protein level may be attributed to stress-mediated immobilization of these compounds to fulfil an increased element of energy by the toxicant was studied by Jenkins and Smith [41]. The depletion of total protein content may be due to augmented proteolysis, and possible utilization of their product for metabolic purposes was reported by Ravinder and Suryanarayana [42].

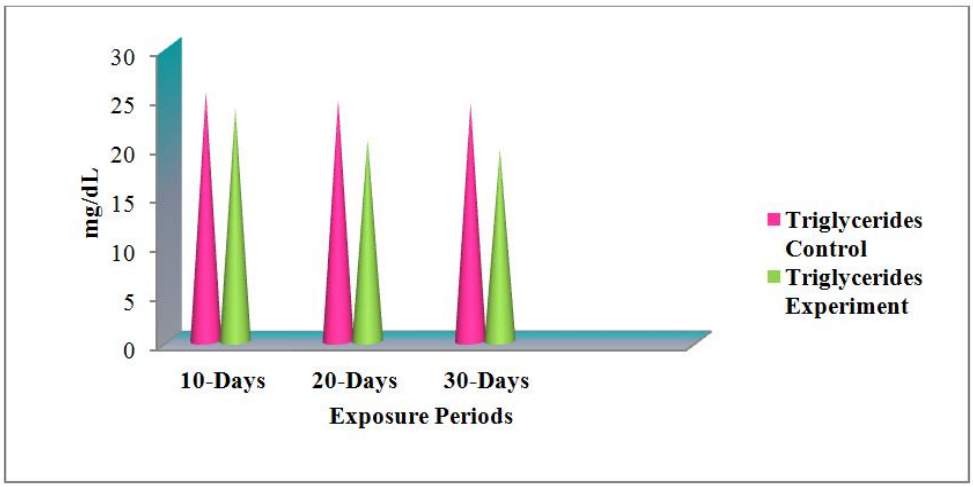

Fig. 7. Showing the changes in the blood of *Catla catla* exposed to 0.42 ppm of Herbicide Glyphosate Hijack® (41%) in long-term duration

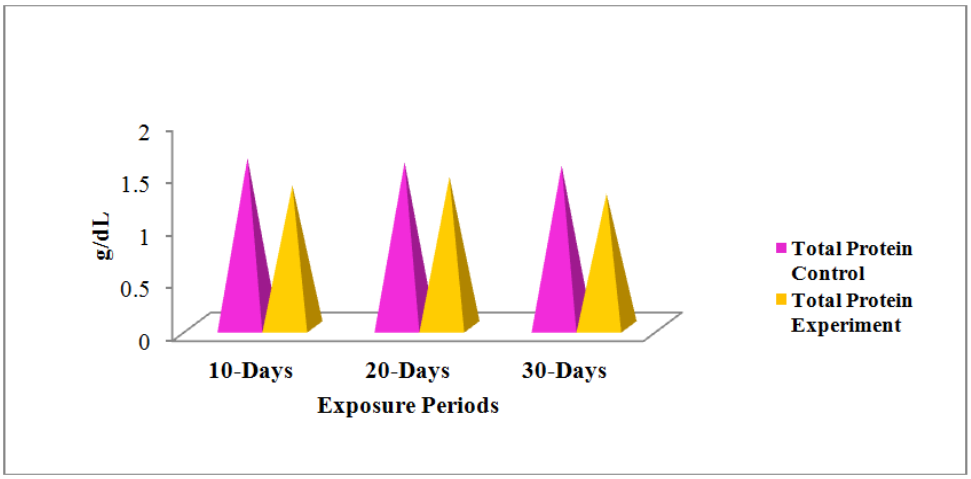

Fig. 8. Showing the changes in the blood of *Catla catla* exposed to 0.42 ppm of Herbicide Glyphosate Hijack® (41%) in long-term duration

4. CONCLUSION

The observations from the present study showed that, these herbicide glyphosate hijack® (41%) at sub lethal concentrations altered the haematological parameters like RBC, WBC, HB, HCT, blood glucose, total cholesterol, triglycerides, and total protein of test fish, due to utilization of biochemical energy to counteract the toxic stress caused due to herbicides present in aquatic media.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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