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Effects of acute exposure of Pendimethalin on some haematological parameters of freshwater fish Channa punctata (Bloch).

Syed Danish[1], Aqib Lone[1], Aafreen Hasan[1], Irfan Parray[1], Jayashree Dhote[2].
[1]Research Scholar, Dept. of Zoology, Shri Shivaji Science College, Amravati, Maharashtra.
[2]Proffessor, Dept. of Zoology, Shri Shivaji Science College, Amravati, Maharashtra.
Email Id: - danish2074@gmail.com

Abstract

In aquatic toxicology, fishes have been employed as recognized forms for studying toxic responses of herbicides. The present study was conducted on acute exposure of herbicide pendimethalin on fresh water fish Channa punctata (Bloch) exposed to sub-lethal doses (1.14 and 2.28 ml/l), for 96 hours. The results when compared to the control group, the fish exposed to pendimethalin showed a significant decrease in Hemoglobin content, number of red blood cells and white blood cell. Glucose content increases significantly which is an indicative response of the organism to the toxicant stress. However, a significant increase in SGPT and SGOT was also recorded with an increase in the exposure period.

Key words: *Haematology, herbicide, Pendimethalin, Channa punctata.*

Introduction

Aquaculture is the fastest-growing food-production sector in the world. Aquaculture supplies 50% of all fish consumed globally today, and it is predicted that by 2030 it will be the prime source of fish. (FAO, 2016). The use and validation of fish health monitoring tools have become increasingly evident due to the expansion of aquaculture. The complete blood cell count (CBC) is an important and powerful diagnostic tool as well as a component of a minimum database. It can be used to monitor the health status of fish in response to changes related to nutrition, water quality and disease an in response to therapy.

The diagnosis of disease using hematological analysis is especially important because it can provide a reliable evaluation via non-lethal means (Satheeshkumar et al., 2012). The complete blood count profile is an important diagnostic tool, with laboratory protocols and reference ranges well established in both human and veterinary medicine. Knowledge and research of hematological parameters can facilitate the development of indicators of the health status of fish in response to changes related to nutrition, water quality and disease. (Noga 2000). The hematological profile of a fish cultivation can indicate its physiological status and health, so that hematology combined with other routine diagnostic methods could be used to identify and assess conditions that cause stress and/or diseases that affect production performance (Tavares-Dias and Moraes, 2007)

The study of the physiological and hematological characteristics of cultured fish species is an essential tool in the advancement of aquaculture systems, especially with respect to the detection of healthy versus infected or stressed fish (Ranzani-Paiva et al., 2003). Blood parameters are useful criteria for showing physiological alterations in intensively farmed fishes and can provide important information for diagnosis and prognosis of diseases. In an aquaculture system, the economic losses can be eliminated when hematological methods are used as aids (early diagnosis) in fish disease diagnostics.

The use of synthetic herbicides has increased with the growing awareness about their utility in agriculture production, animal husbandry, postharvest technology and in the public health and welfare of mankind. The intensive use of synthetic herbicides in agricultural fields and public health operation systems has resulted in serious environmental hazards (Singh 2004). Due to their long term persistence, slow degradability in the water, toxicity to other organisms (Arasta et al., 1996) and accumulation inside the fish body, synthetic herbicides adversely affect the aquatic environment (Cullen and Connell 1992). To minimize environmental pollution by pesticides, efforts are being made to find herbicides from plant origin because plants are virtually inexhaustible source of structurally diverse and biologically active substances (Mian and Mulla 1992).

Pendimethalin is a di-nitroniline herbicide (N-(1-ethylpropyl)-3, 4-dimethyl-2, 6-dinitro-benzenamine; Pendimethalin). The empirical formula for pendimethalin is C13H19N3O4 is a herbicide used to control annual grasses and certain broadleaf weeds in agricultural fields (Bandyopadhyay and Choudhury 2009). The compounds can also bio-accumulate and bio-magnify, bio-concentrating up to 70,000 times their original concentrations (Solomon et al., 2007). Pendimethalin, a herbicide, contaminates aquatic bodies mostly through runoff and spray drift (Danion et al., 2012). Fish and aquatic invertebrates are very hazardous to pendimethalin (Meister., 1992). In the fish Channa Punctatus, this chemical caused haematological changes.

Materials and Methods

COLLECTION OF FISH AND MAINTENANCE

Channa punctata specimens were gathered in good health near Amravati from the local 'Wadali lake.' Fish of about 15-20 cm in length and weighing between 60 and 80 gramme were used in the experiment. Fish were given minced goat liver at a rate of 3% body weight ad libitum during the acclimatization phase. The acute toxicity tests were carried out in rectangular glass aquaria with a capacity of 40 liters; feeding was discontinued for 24 hours previous to the commencement of the tests. The bottom of the container was flat. They were easy to clean, and there was no clutter in the corners or cracks. They were rinsed twice with tap water before each experiment, cleaned with detergent, rinsed once with 10% HCl, and then washed twice with tap water..

Group I - Served as control or normal group.

Group II - Treated or experimental group.

3.5 ACUTE TOXICITY-LC50

The average mortality in each concentration was used to determine the LC50 in Channa punctata by showing a graph with concentration on the X-axis and mortality on the Y-axis. The LC50 was determined using Finnyl's Probit analysis. According to graphical plots, the 50% mortality values of pendimethalin for 96 hours were 11.4 mg/L. For 24, 48, 72, and 96 hours, these fish were administered an acute dosage of pendimethalin (1/5 = 2.28 mg/L).

HAEMATOLOGY

Blood samples were taken from the control and experimental fish. Approximately 2 mL of blood was collected from the caudal peduncle using separate heparinized disposable syringes containing 0.5 mg ethylate diamine tetra acetic acid (EDTA) as anticoagulant; it was properly mixed and used for haematological analysis. All haemtological parameters such as, haemoglobin content, total leukocyte count, erythrocyte count and blood biochemistry such as serum glucose,

haemoglobin (Hb) concentration values were determined by the microhematocrit capillary tube and cyanomethaemoglobin methods (Hesser, 1960).

Red blood cell (RBC), and total white blood cell (WBC), were evaluated using the Neubauer haemocytometer (Shah, 2010).

SGPT activity was assayed by using SGPT assay kit (ADG-PT 50) through the Optimised IFCC Method.

The activity of SGOT was determined using the Optimised (IFCC Method 1986) and SGPT assay kits (ADG-OT 50).

Results and Discussion

Sublethal concentration (mg/l)	Control	Experimental Groups			
		24 Hours	48 Hours	72 Hours	96 Hours
2.28	4.19 ± 0.30	4.09 ± 0.22	4.01 ± 0.17	3.83 ± 0.10	3.62 ± 0.14
1.14	4.28 ± 0.21	4.15 ± 0.24	4.11 ± 0.18	4.03 ± 0.24	3.94 ± 0.18

Table: 1. Effect of lower and higher sublethal concentrations of pendimethalin on Total erythrocyte count of fish Channa punctatus.

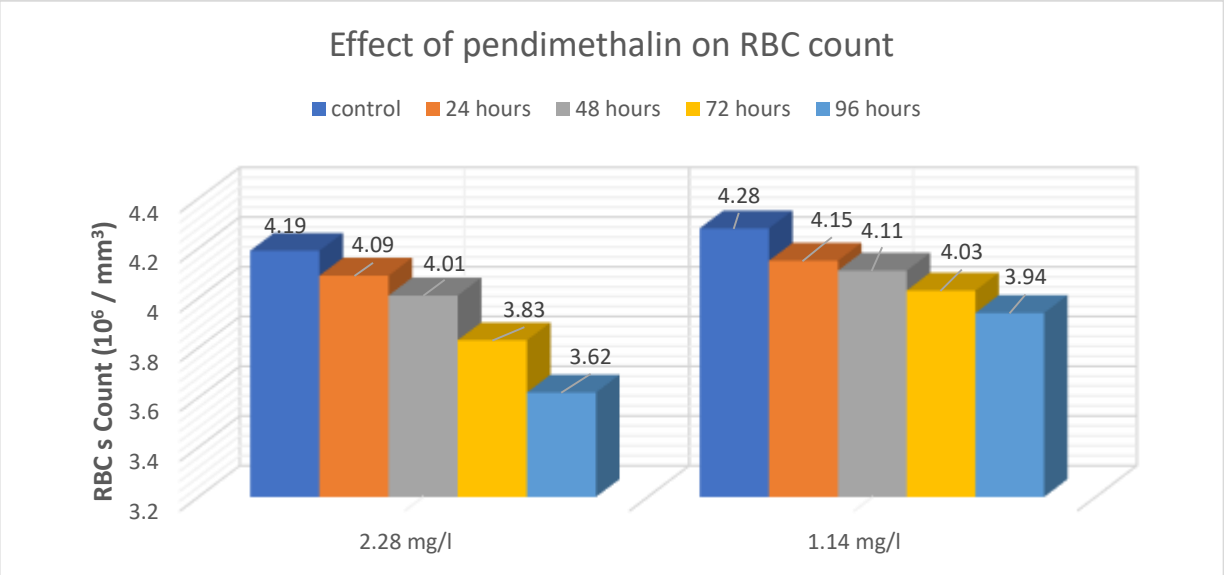

Figure 1. Graphical representation of Total erythrocyte count of fish Channa punctatus exposed to lower and higher sublethal concentration of pendimethalin.

Sublethal concentration (mg/l)	Control	Experimental Groups			
		24 Hours	48 Hours	72 Hours	96 Hours
2.28	7.15 ± 0.21	7.02 ± 0.34	6.84 ± 0.21	6.51 ± 0.41	6.31 ± 0.26
1.14	7.24 ± 0.29	7.12 ± 0.24	6.94 ± 0.09	6.88 ± 0.21	6.71 ± 0.14

Table: 2. Effect of lower and higher sublethal concentrations of pendimethalin on Total Leucocytes count of fish Channa punctatus.

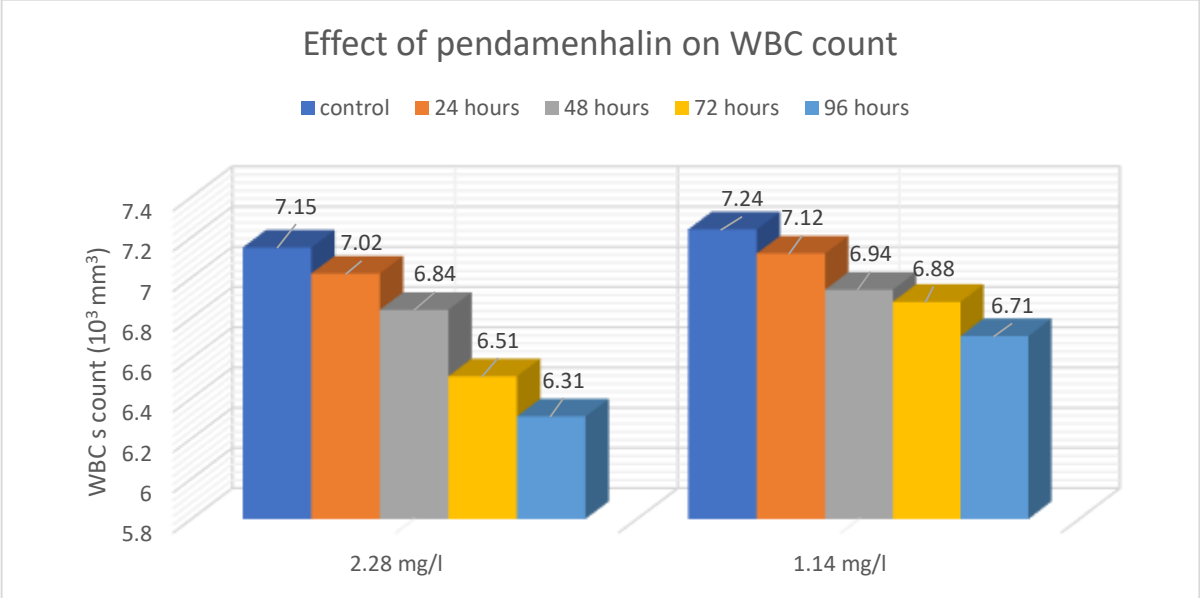

Figure 2. Graphical representation of Total Leucocytes count of fish Channa punctata exposed to lower and higher sublethal concentration of pendimethalin.

Sublethal concentration (mg/l)	Control	Experimental Groups			
		24 Hours	48 Hours	72 Hours	96 Hours
2.28	13.14 ± 0.42	13.04 ± 0.24	12.81 ± 0.46	12.52 ± 0.41	11.91 ± 0.53
1.14	13.08 ±0.34	13.02 ± 0.50	12.98 ± 0.19	12.76 ± 0.17	12.71 ± 0.23

Table: 3. Effect of lower and higher sublethal concentrations of pendimethalin on Haemoglobin content of fish Channa punctatus.

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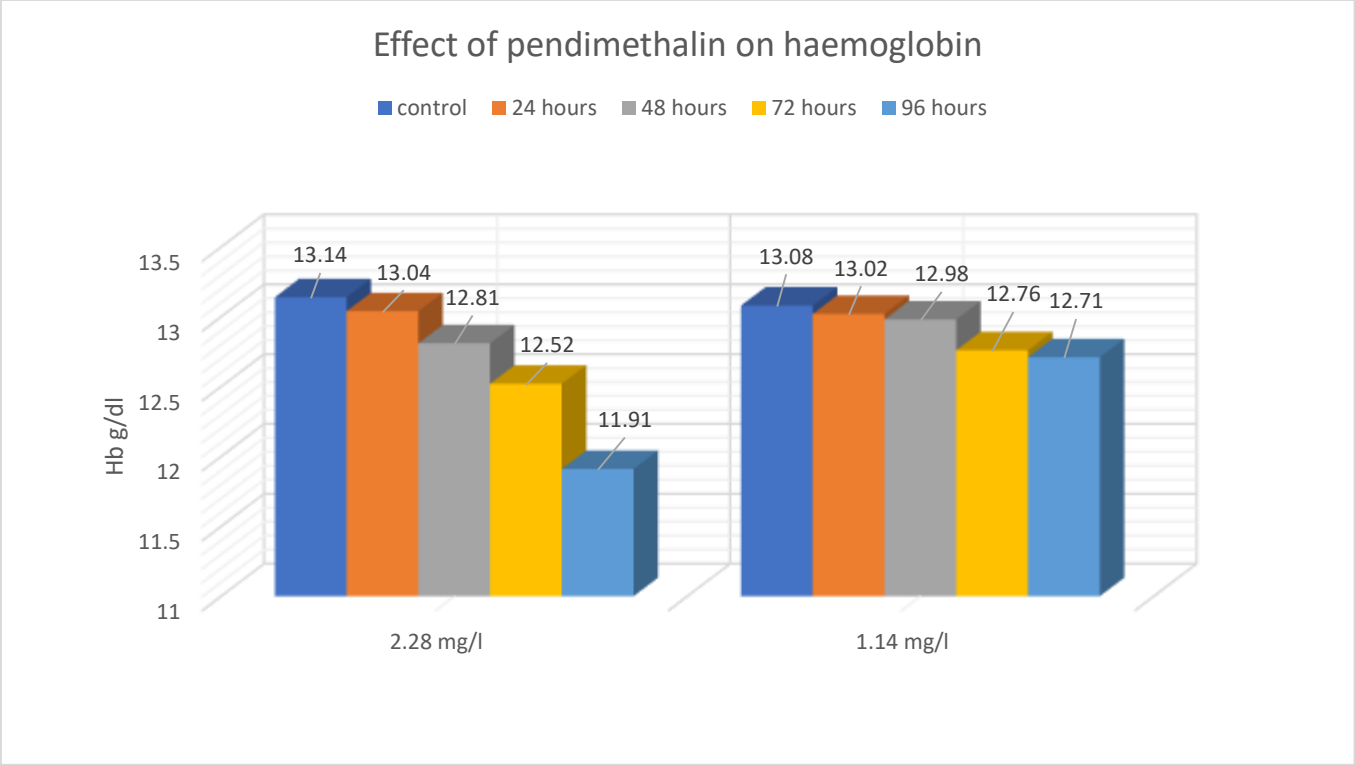
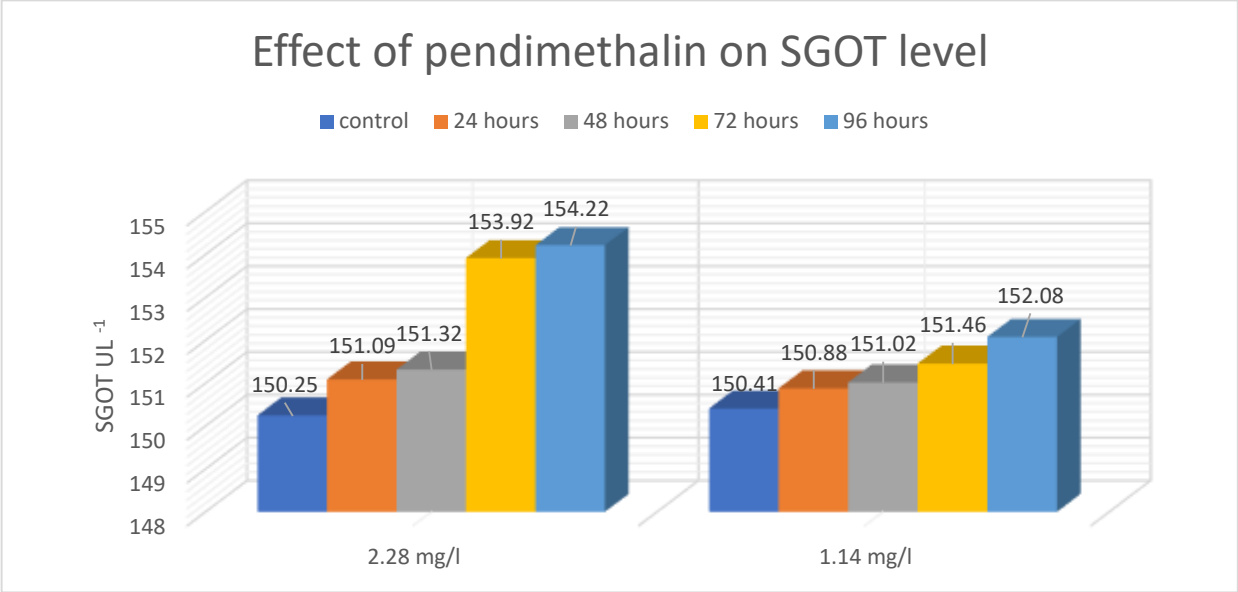


Figure 3 . Graphical representation of heamoglobin content of fish Channa punctatus exposed to lower and higher sublethal concentration of pendimethalin

Sublethal concentration (mg/l)	Control	Experimental Groups			
		24 Hours	48 Hours	72 Hours	96 Hours
2.28	150.25 ± 1.93	151.09 ± 2.61	151.32 ± 3.49	153.92 ± 2.39	154.22 ± 3.10
1.14	150.41 ± 2.44	150.88 ± 2.42	151.02 ± 1.68	151.46 ± 2.42	152.08 ± 2.09

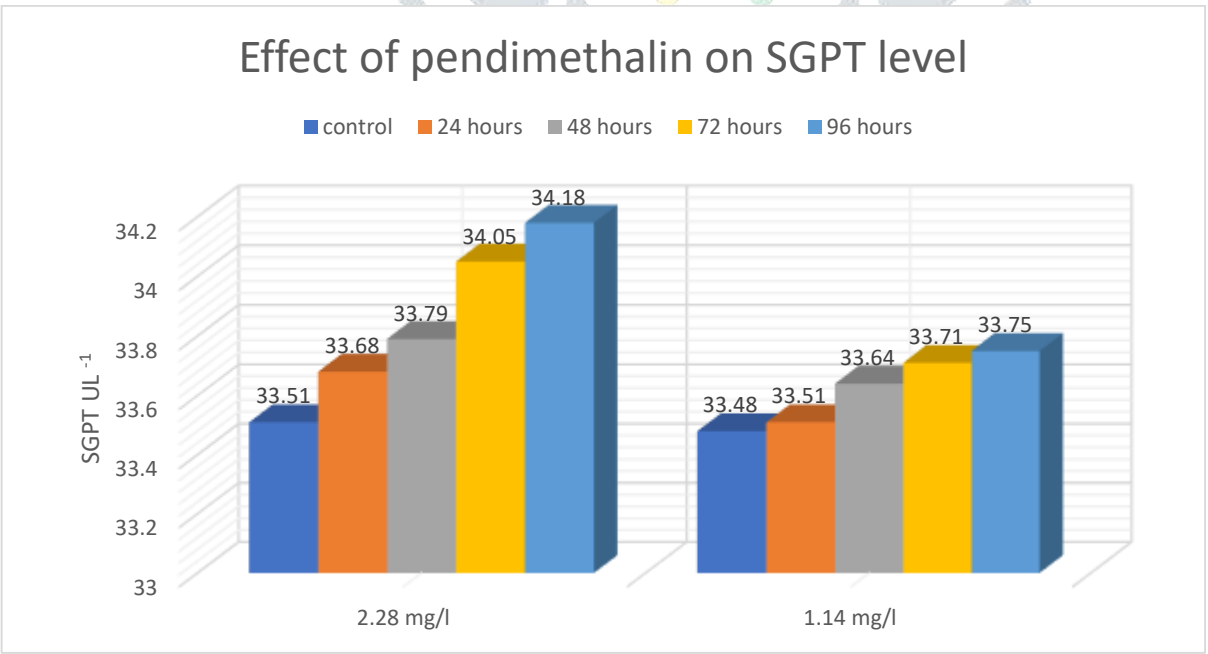
Table 4: . Effect of lower and higher sublethal concentrations of pendimethalin on SGOT levels of fish Channa punctatus.



Graph 4: Graphical representation of SGOT level of fish Channa punctatus exposed to lower and higher sublethal concentration of pendimethalin.

Sublethal concentration (mg/l)	Control	Experimental Groups			
		24 Hours	48 Hours	72 Hours	96 Hours
2.28	33.51 ± 0.72	33.68 ± 0.41	33.79 ±0 .88	34.05 ± 0.64	34.18 ± 0.77
1.14	33.48 ± 0.54	33.51 ± 0.76	33.64 ± 0.53	33.71 ± 0.46	33.75 ± 0.43

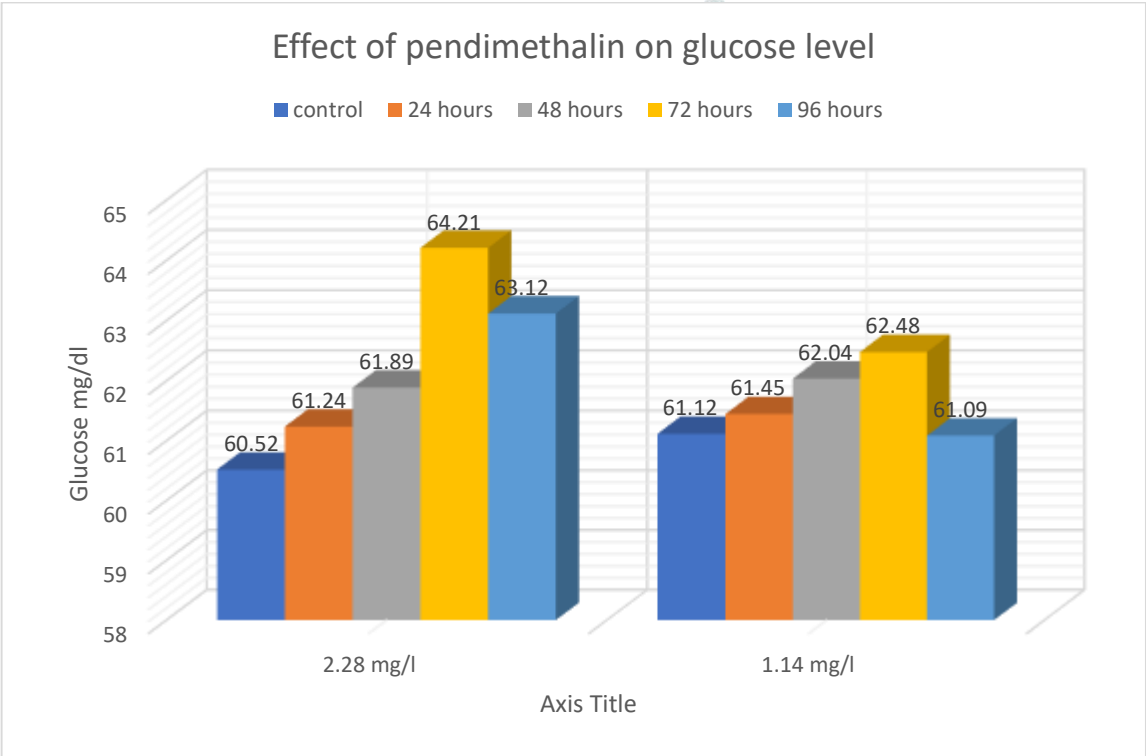
Table 5: Effect of lower and higher sublethal concentrations of pendimethalin on SGPT levels of fish Channa punctatus.



Graph 5: Graphical representation of SGPT level of fish Channa punctatus exposed to lower and higher sublethal concentration of pendimethalin.

Sublethal concentration (mg/l)	Control	Experimental Groups			
		24 Hours	48 Hours	72 Hours	96 Hours
2.28	60.52 ± 1.42	61.24 ± 2.11	61.89 ± 1.74	64.21 ± 2.10	63.12 ± 1.96
1.14	61.12 ± 1.54	61.45 ± 1.89	62.04 ± 1.59	62.48 ± 1.17	61.09 ± 1.64

Table 6: Effect of sublethal concentrations of pendimethalin on glucose levels of fish Channa punctatus.



Graph 6: Effect of sublethal concentrations of pendimethalin on glucose levels of fish Channa punctatus.

4.4.1. Total erythrocyte count (TEC)

The total erythrocyte count (x106mm-3) of fishes exposed to Pendimethalin was presented in Table 1 and plotted a graph in the Figure 1. A significant (P<0.01) decline of total erythrocyte count was observed in both the higher and lower concentration on the 96-h of exposure. In lower concentration at the end of 96 hours of exposure the mean value of TEC was significantly (P<0.05) reduced compare with that of control. The decreasing pattern was higher in high concentration and lower in lower concentration when compared with control.

4.4.2. Total leucocyte count (TLC)

The total leucocyte count (x103mm-3) of fishes exposed to Pendimethalin was presented in Table 2 and plotted a graph in the Figure 2. A significant reduction (P<0.01) was found in the mean value of TLC in both

experimental groups at the experimental period of the fishes exposed to the herbicide. In both the concentration at the end of 96 hours of exposure the mean value of TLC was decreased significantly (P<0.05) when compared to control.

Hemoglobin content:

The total hemoglobin content (Hb gm/Dl) of fishes exposed to Pendimethalin was presented in Table 3 and plotted a graph in the Figure 3. A significant reduction (P<0.01) was found in the mean value of hemoglobin in both experimental groups at the experimental period of the fishes exposed to the herbicide. In both the concentration at the end of 96 hours of exposure the mean value of hemoglobin was decreased significantly (P<0.05) when compared to control.

SGOT and SGPT

The SGOT and SGPT levels Ul^{-1} are depicted in table 4 and 5, the results shown increase in enzymatic levels.

Discussion

In the present investigation, it has been observed that low level sublethal exposure of pendimethalin affects haematology. (Omoregie et al.1990) reported that toxicants and pollutants have significant effects which can result in several physiological dysfunctions in fish. Dysfunction in the fish induces changes in blood parameters. In fishes, a change of the blood cell distribution is also correlated with the changes in environmental conditions. The exposure of Channa punctatus to sublethal concentrations of pendimethalin caused a significant decrease in erythrocyte count, haemoglobin and leucocyte count of the fish. The decrease in haemoglobin concentration is similar to those reported in C. gariepinus exposed to cassava effluents and tobacco (Nicotiana tobaccum Linn.) leaf extracts (Adeyemo 2005). This pattern of response may be attributed to haemolysis.

The decrease in number of erythrocytes might have been achieved through failure or suppression of normal mechanisms promoting erythropoiesis and/or deficiency of some factors required for the maturation of the red cell. Thus, the significant reduction in these parameters is an indication of severe anaemia.

The white blood cells in fish respond to various stressors including infections and chemical irritants (Christensen and Faindt-Poeschi 1978). Thus, increasing or decreasing numbers of white blood cells are a normal reaction on the exposure of toxicants (Kori-Siakpere et el., 2006). In the present investigation the decrease in WBC (leukocytosis) may have resulted from of defense mechanism of the fish to counter the effect of the toxicant.

Blood glucose has been employed as an indicator to environmental stress. (Silbergeld 1974) The fluctuation in glucose is the cause of stressed environment.

The elevated levels of serum transaminases (SGOT and SGPT) are markers of liver functions, that were observed in Channa punctatus. This increased level of serum transaminases related to disruption of normal metabolism which is due to extensive alterations in the liver histology and indicates liver damage.

CONCLUSION

The findings stated that Pendimethalin is toxic and also induced heamatological alteration in fish Channa punctatus.because of its wide use in agriculture and unavoidable contact with humans. Utmost precaution should be taken while handling pendimethalin.

So, this study strongly recommends practice of more concerns during pesticide usage and more attention is required to be paid to the selection of appropriate bioindicator for evaluation of toxic effects of xenobiotics.

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