



EFFECTS OF CYPERMETHRIN ON HAEMATOLOGICAL MARKERS IN WALLAGO ATTU

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Abstract: *Wallago attu* was exposed to five different concentrations of cypermethrin for 30 days and found a remarkable change in haemoglobin, TLC, PCV and TEC values. A surging rise was noticed in all the four parameters on exposure to the different concentration of cypermethrin on day 1 but there was a slight reduction in the haemoglobin content, PCV and TEC with a slight rise in TLC after few days. *Wallago attu* exposed to cypermethrin showed an increase in blood glucose level. Cypermethrin has potent piscicidal activity against *Wallago attu* and adversely affects.

Keywords: Cypermethrin, Haematology, Pesticides, *Wallago attu*.

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INTRODUCTION

Pesticides are the biological toxicants, which are being used by the man to kill the pests for increasing the yield of many crops and insect vectors to control the spread of disease. The use of pesticides has caused severe environmental and health hazards to organisms including human beings (Prakash and Verma, 2014). The widespread use of pesticides not only brought adverse influence on agro ecosystems but also caused alteration in the ecological balance of many non-target organisms like fishes (Masih, 2021). The excessive use of pesticides is an anthropogenic event that causes severe biodiversity threats (Prakash and Verma, 2022). The pesticides through surface runoff reach into the aquatic ecosystems and become a global environmental problem. These pesticides enter

the food chain and their subsequent bioaccumulation and biotransformation at different trophic levels have catastrophic effect to the ecosystem (Grande *et al.*, 1994). During the last two decades a tremendous progress has been made in the development of new compounds with better toxicity, therefore, a lot of works have been carried out on impact of pesticides on non-target aquatic organisms. The most of synthetic organic pesticides are extremely toxic to non-target species of freshwater fauna, which damage the population dynamics, complex food-web and food-web energetic (Prakash, 2020). Verma and Prakash (2018a and 2018b) also worked a lot on pesticide effect on different fresh water fishes.

Cypermethrin is a synthetic pyrethroid used as an insecticide to control ectoparasites which infest



cattle, sheep, poultry and some companion animals on a large scale (Krishna and Prakash, 2015). Cypermethrin is highly toxic to fish, bees, and aquatic insects. It is found in many household ants and cockroach killers, including ant chalk. Cypermethrin is more persistent under anaerobic conditions. Fishes are particularly poisonous to pyrethroid pesticides, with 96-hour LC₅₀ values typically sub 10 g/l. In birds and other animals, the LD₅₀ values are larger (hundreds to thousands of mg/kg). Fishes are more sensitive to pyrethroids because of their slow metabolism and delayed elimination (Bradbury and Coats, 1989). It photodegrades rapidly with a half-life of 8 to 16 days. Several workers studied the consequences of pesticides on different parameters of different haematological parameters of fishes including Kaur and Mishra (2019). Exposure to varying periods and concentrations of pesticides brings substantial changes in blood haemoglobin %, TLC, TEC and packed cell volume. Gopal *et al.* (1982) observed that a low concentration of endosulfan increases TEC while its higher concentration lowers TEC concentration. Identical results were also found within haemoglobin %. Because of these facts, it appeared worthwhile to study the effect of exposing a freshwater fish *Wallago attu* to different concentrations of cypermethrin at different time intervals.

MATERIALS AND METHODS

Wallago attu (35±5.01 g and 15±5.06 cm) was collected from the local fish market, Balrampur, and transported to the laboratory. The fish were treated with 0.1 % KMNO₄ solution to get rid of dermal infection and acclimatized for 15 days to the laboratory conditions, during which they were fed with commercial floating fish feed and the water in the tank was changed regularly to remove faeces and food remnants. Water parameters were monitored as per APHA (2005). The temperature of 23-25 ° C, pH 7.3-7.8 mg / l,

and dissolved oxygen 7.0 -7.5 were maintained. After acclimatization, fishes were transferred to 0.0015, 0.003, 0.006, 0.008 and 0.01 ppm of cypermethrin which were approximately 10,20, 40, 50 and 63% of LC₅₀/96h being 0.016 ppm. The fishes were reared in these concentrations for 1,3,7,15,21 and 30 days in groups of 10 each. Controls were maintained in tap water. No food was given during the experimental period. Fish were anaesthetized after 7 days and 14 days of both groups with 0.2 mL/L of clove mL/L of clove oil in <3 min than blood samples was taken from each fish by puncturing the caudal vein using 1 mL tuberculin syringes. The collected blood was used to determine TEC and TLC with Neubauer's counting chamber and Neubauer's counting chamber and Neubauer's hemocytometer, using Toission's solution as diluting fluid for TEC and Turk's solution for TLC. Haemoglobin concentration and PCV were determined by standard methods. The volume of the blood collected in each case was measured in finely graduated tubes. Blood was drawn from the caudal peduncle and examined right away to determine glucose levels (mg/dL). Using glucose strips, the Accu-Chek GHb, blood glucose/haemoglobin dual-function monitoring system, assessed blood glucose (mg/dL).

RESULTS AND DISCUSSION

As a result of cypermethrin exposure to *Wallago attu* on day 1, TEC rose dramatically for all the five concentrations. The climb lasts for a few days after that, but on day 30, the values are lower than any of the previous days' maximum TEC. Additionally, the significant increase on day 1 is related to the concentration of the pesticides (about 7.00, 11.90, 28.91, 69.40 and 115.0% over the control with five successive concentrations) (Table 1 and Fig. 1.). The fall in TEC concentration after continued exposure, as perceived on day 30, TEC was in the highest concentration of cypermethrin.

Table 1: Effect of cypermethrin on Total Erythrocyte Count (million /mm³) in *Wallago attu*.

Cypermethrin conc. (ppm)	Exposure period						
	0 Day	1 Day	3 Days	7 Days	15 Days	21 Days	30 Days
0.0015	3.04±0.16	3.18±0.20	3.22±0.23	3.67±0.05	3.69±0.74	3.21±0.38	2.85±0.01
0.003	2.44±0.26	2.51±0.28	3.38±0.33	3.30±0.75	2.95±0.19	3.09±0.60	3.51±0.90
0.006	2.68±0.44	3.15±0.15	3.66±0.11	4.06±0.69	3.56±0.18	3.50±0.24	3.63±0.31
0.008	2.80±0.21	4.43±0.32	4.37±0.09	5.15±0.22	5.38±0.12	4.77±0.42	4.51±0.50
0.01	2.69±0.41	4.98±0.17	5.67±0.10	5.66±0.04	5.45±0.37	5.06±0.33	4.73±0.29

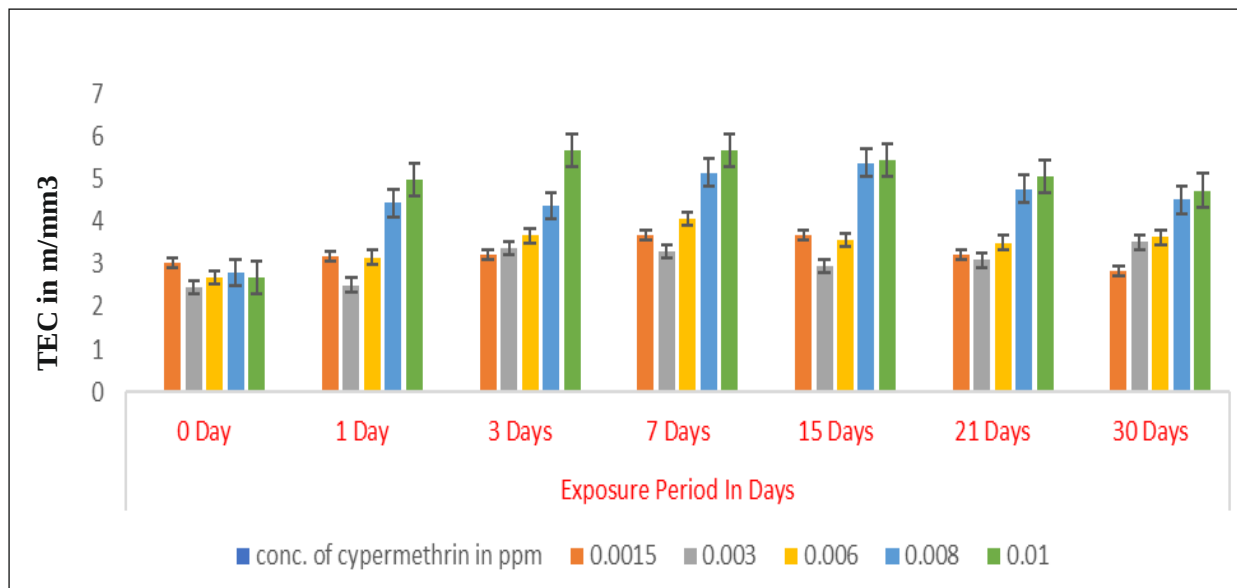


Fig 1: Effect of cypermethrin on Total Erythrocyte Count in *Wallago attu*.

The TLC calculation comprised thrombocytes, lymphocytes, basophils, neutrophils, eosinophils, blast cells, and smudge cells. TLC changes closely resemble TEC changes after exposure to cypermethrin (as shown in Table 2 and fig. 2). On day 1, there is a notable increase in TLC, which increases with cypermethrin

concentration, though not as much as the increase with the highest TEC concentration. Furthermore, unlike TEC, TLC grows steadily from day 15 to day 30 in the three higher concentrations, despite some decline in the two lower concentrations which is more significant in the lowest.

Table 2: Effect of cypermethrin on Total Leucocyte Count (thousands per mm³) in *Wallago attu*.

Conc. of Cypermethrin in (ppm)	Exposure period						
	0 Day	1 Day	3 Days	7 Days	15 Days	21 Days	30 Days
0.0015	44.66±1.23	51.25±1.38	53.67±2.06	57.49±1.68	26.54±0.61	55.62±0.65	53.08±0.19
0.003	37.84±1.76	48.13±1.87	51.72±1.02	50.25±1.20	55.96±0.44	56.22±0.78	53.34±1.21
0.006	42.38±1.81	58.04±1.64	65.66±1.52	61.60±1.90	59.72±0.18	64.36±0.47	64.61±1.71
0.008	45.22±0.84	65.47±1.33	59.34±1.27	68.63±0.22	62.77±0.11	65.88±0.29	67.16±2.20
0.01	41.74±1.22	63.40±0.86	73.72±0.94	69.46±1.73	67.88±1.11	69.06±1.56	69.77±1.18

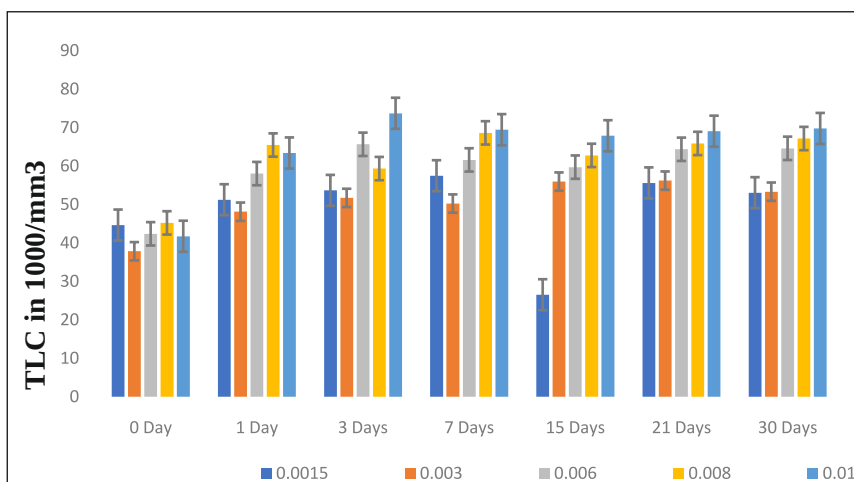
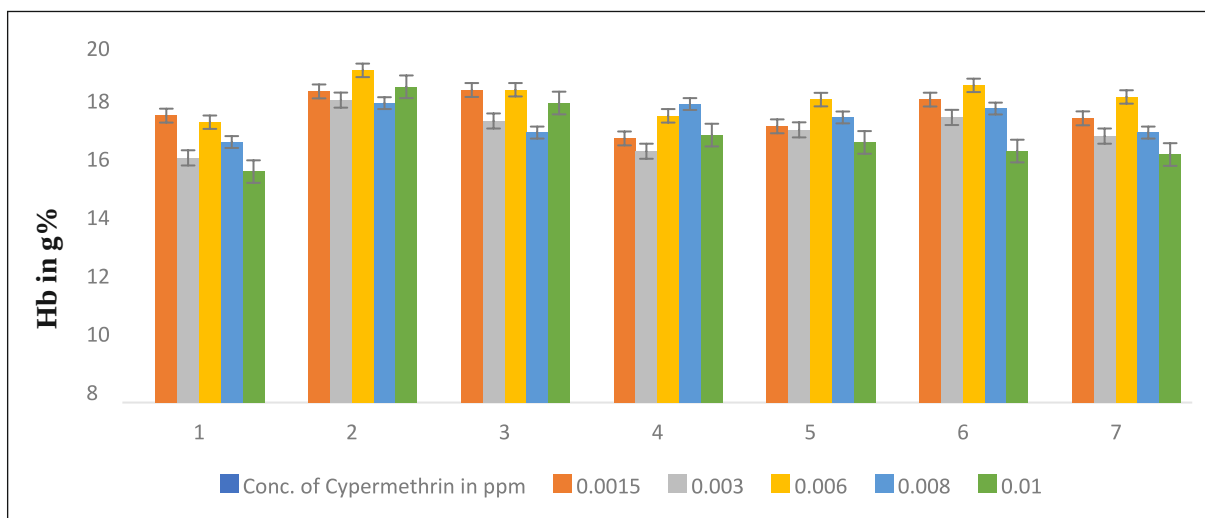


Fig 2: Effect of cypermethrin on Total Leucocyte Count in *Wallago attu*.

Table 3: Effect of cypermethrin on Haemoglobin concentration (g%) in *Wallago attu*.

Cypermethrin Conc. (ppm)	Exposure period						
	0 Day	1 Day	3 Days	7 Days	15 Days	21 Days	30 Days
0.0015	16.10±1.86	17.46±1.23	17.54±2.23	14.82±1.12	15.50±1.60	17.00±1.30	15.95±2.011
0.003	13.73±1.44	16.97±1.43	15.80±2.08	14.10±2.15	15.30±1.10	16.00±0.93	14.95±1.80
0.006	15.73±1.77	18.64±1.77	17.55±1.22	16.09±1.15	17.00±1.80	17.80±1.05	17.15±1.55
0.008	14.62±1.42	16.80±1.21	15.15±1.68	16.75±1.09	16.00±1.11	16.50±1.15	15.15±1.12
0.01	12.96±1.50	17.72±1.34	16.81±1.71	15.01±1.11	14.60±0.99	14.11±1.07	13.92±1.17



3: Effect of cypermethrin on Haemoglobin percentage in *Wallago attu*.

TEC and TLC, as well as variations in haemoglobin percentage (Hb percent) (Table 3 and Figure 3) and PCV (Table 4 and Figure 4) are identical. On the first day, both Hb percent and PCV levels rise dramatically, and this rise is connected to pesticide levels. On days 7 and 3, the maximum values are obtained at the two

greatest concentrations of 0.008 and 0.01 ppm, respectively, and gradually fall, with the values on day 30 being lower than those on day 1. The percentage increase in haemoglobin ranges from 5.5 on day 1 to 53.7 on day 30, while PCV rises from 6.9 to 35.7. It is toxic, even at the lowest level of 0.0015 ppm.

Table 4: Effect of cypermethrin on PCV (%) in *Wallago attu*.

Cypermethrin conc. (ppm)	Exposure period						
	0 Day	1 Day	3 Days	7 Days	15 Days	21 Days	30 Days
0.0015	16.10±1.86	17.46±1.23	17.54±2.23	14.82±1.12	15.50±1.60	17.00±1.30	15.95±2.011
0.0015	55.10±0.10	57.40±1.01	60.50±1.15	60.10±1.30	60.00±1.16	61.41±0.08	63.70±2.01
0.003	48.20±1.21	59.20±1.39	57.30±1.14	61.60±1.81	57.30±1.12	58.30±1.12	57.90±1.89
0.006	51.90±1.06	62.50±1.16	61.20±0.07	63.30±1.19	64.90±1.06	64.90±1.61	61.90±1.34
0.008	54.20±1.33	67.80±1.71	66.40±1.29	68.50±0.69	63.20±1.03	63.20±1.91	62.80±1.66
0.01	52.40±1.29	67.90±1.61	69.60±1.69	66.90±0.09	61.80±1.20	60.80±1.99	60.10±1.40

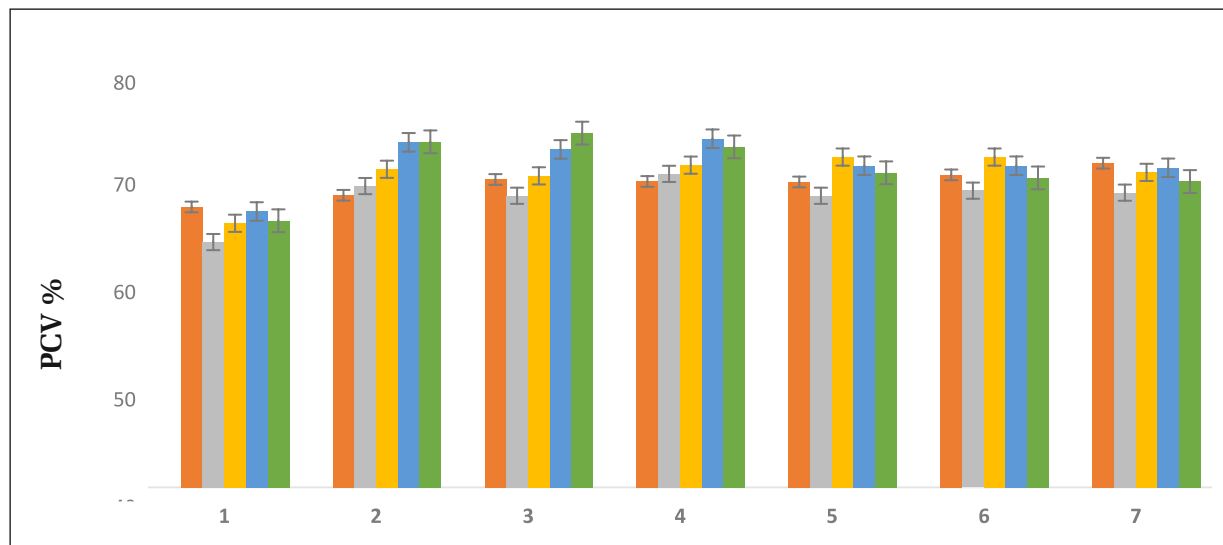


Fig 4: Effect of cypermethrin on PCV in *Wallago attu*.

On different days, the average volume of blood obtained from fish treated with varying concentrations of cypermethrin. Each instance

had a smaller blood volume than the controls, and the decline is linked to the cypermethrin content (Table 5; figure 5).

Table 5: Effect of cypermethrin on the volume of blood (ml/100 g body weight) collected from *Wallago attu*.

Cypermethrin conc. (ppm)	Exposure period						
	0 Day	1 Day	3 Days	7 Days	15 Days	21 Days	30 Days
0.0015	2.61	2.50	1.81	2.23	1.89	1.95	1.65
0.003	3.20	2.87	2.10	2.62	2.38	1.80	2.15
0.006	2.80	2.29	1.51	1.55	2.65	2.59	2.79
0.008	2.90	2.33	1.78	1.51	1.23	1.10	1.22
0.01	2.96	2.80	1.89	1.72	1.55	1.63	1.41

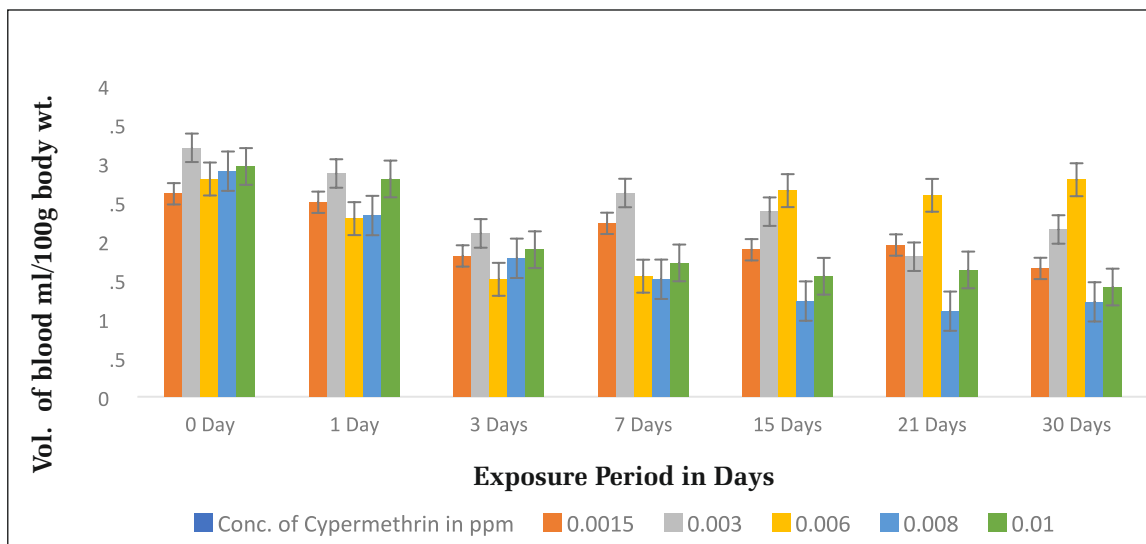


Fig 5: Effect of cypermethrin on the volume of blood collected from *Wallago attu*.

The fact that all four parameters investigated, TEC, TLC, Hb percent, and PCV, showed a considerable increase in their values on day 1 is important, and the increase is related to the concentration of cypermethrin. TEC is 7 % above control on day 1 at 0.0015 ppm and 115.0 percent above control at 0.01 ppm. TLC is 17.75 % and 57.10 % greater than the control, Hb is 5.47 percent and 53.80 percent higher than the control, and PCV is 6.9% and 35.7 % higher than the control. It's also worth noting that, with the exception of TLC, all three indicators achieve their maximum during the first two weeks and then begin to decline. This is particularly apparent at higher pesticide concentrations. After day 15, TLC, on the other hand, grows.

TEC, TLC, Hb percent, and PCV have all increased in diverse fishes, according to several researchers. Organophosphorus insecticides, carbamates, and even metal toxicity have all been linked to a large increase in these parameters. However, some employees see a decrease in these parameters when exposed to certain toxicants. Polycythemia was caused by a lower concentration of organophosphate, while erythropenia was caused by a higher dose. Lower Cypermethrin concentrations cause TEC to drop, whereas higher concentrations cause it to rise. Torres-Rosas *et al.* (2014) proposed that under stress, neuronal components connected with blood vessels and stroma are stimulated, promoting haemopoiesis..

Pesticides, according to Bansal *et al.* (1979), promote haemoconcentration, which raises the value of TEC per unit volume without increasing TEC. TLC has been linked to an increase in thrombocytes, lymphocytosis, and WBC squeezing in the peripheral blood. In the case of Hb, pesticides are expected to have a catalyzing effect on the incorporation of body iron reserves into Hb, in addition to the effect of corpuscle shrinkage. The increase in PCV values of fish treated with toxicants could possibly be due to a malfunctioning osmoregulation mechanism. In fact, aggregation of RBC in the spleen and kidney is commonly thought to imply haemopoiesis, which could be induced by injury to capillaries and corpuscle escape (Kumar and Pant, 1985).

Pesticides have a variety of physiological and histopathological consequences. Most crucial, insects' first physiological response to insecticides is a quick loss of water. Furthermore, because the quality of pesticides in the blood or tissues seldom reaches a substantial level, this response is most likely owing to their effects on CNS regulatory centres, which are triggered by nerve impulses. Toxic substances influence the regulatory centres of the hypothalamus in fish, causing similar water loss. A rise in the amounts of electrolytes in pesticide-treated fish indicates that such a loss occurs.

Throughout the experiment, the volume of blood obtained remains low, yet the values of these factors remain high. As a result, it is possible to conclude that haemoconcentration, which arises from water loss under pesticide stress, is a major, if not the most important, element explaining the per unit increase in TEC, TLC, Hb, and PCV, at least in the early stages of the experiment. The decrease in TEC after day 3 or day 7 in a higher concentration of cypermethrin could be due to the pesticide's negative influence on the haemopoietic process, while the increase in TLC after day 15 in the three higher concentrations could represent the fish's response to the pesticide's pathology.

In the current investigation, cypermethrin therapy resulted in a considerable increase in TLC in *Wallago attu*. *Channa punctatus* has shown similar results after being exposed to eldrin and endosulfan (Abidi and Srivastava, 1988). The main reason of the increase in TLC has been identified as hemoconcentration. A decline in non-specific immunity of the fish can be defined as changes in both erythrocyte and leukocyte counts after exposure to cypermethrin and carbofuran (Svoboda *et al.*, 2001).

The changes in these blood indices could be due to a defence reaction against cypermethrin toxicity by stimulating erythropoiesis, or they could be due to disturbances in both metabolic and haemopoetic activities of fish exposed to below safe cypermethrin concentrations, according to the current study. Hematological

disruption was created by the toxicant, which could damage the fish's ability to fight disease, diminish its odds of survival, and limit its growth and reproduction potential.

The improvement in blood parameters of the *Wallago attu* after 30 days of exposure to cypermethrin- free waters revealed that cypermethrin did not build in the body and was slowly removed, resulting in recovery from pesticide toxicity. Adhikari *et al.* (2004) investigated the effects of cypermethrin and

carbofuran on haematological parameters and recovery in *Labeo rohita* and found similar results.

Environmental stress has been demonstrated to be a sensitive indicator of blood glucose levels. Increased glucose levels in fish exposed to different concentrations of cypermethrin (in ppm) and high temperatures may be related to the mobilization of glycogen into glucose to meet the increased demand for energy used in countering the stress generated by high temperatures in the current investigation.

Table 6: Effect of cypermethrin on the blood glucose (mg/100 g) in *Wallago attu*.

Conc. of Cypermethrin in ppm	Exposure period						
	0 Day	1 Day	3 Days	7 Days	15 Days	21 Days	30 Days
0.0015	33.74±1.42	45.90±1.09	57.73±1.60	38.55±1.19	58.47±1.54	44.78±1.17	59.69±1.20
0.003	34.47±2.89	47.15±1.51	58.10±1.93	39.05±2.91	59.08±1.21	45.15±1.42	61.19±1.09
0.006	35.69±2.09	48.25±1.98	59.63±1.21	40.91±1.10	60.06±2.09	46.11±1.12	62.06±1.77
0.008	36.15±4.85	49.16±2.60	60.15±4.01	41.92±2.15	61.74±2.87	47.90±1.62	63.11±1.36
0.01	39.69±3.01	50.51±1.15	62.69±2.09	46.00±1.18	63.69±2.22	51.69±1.89	65.04±1.29

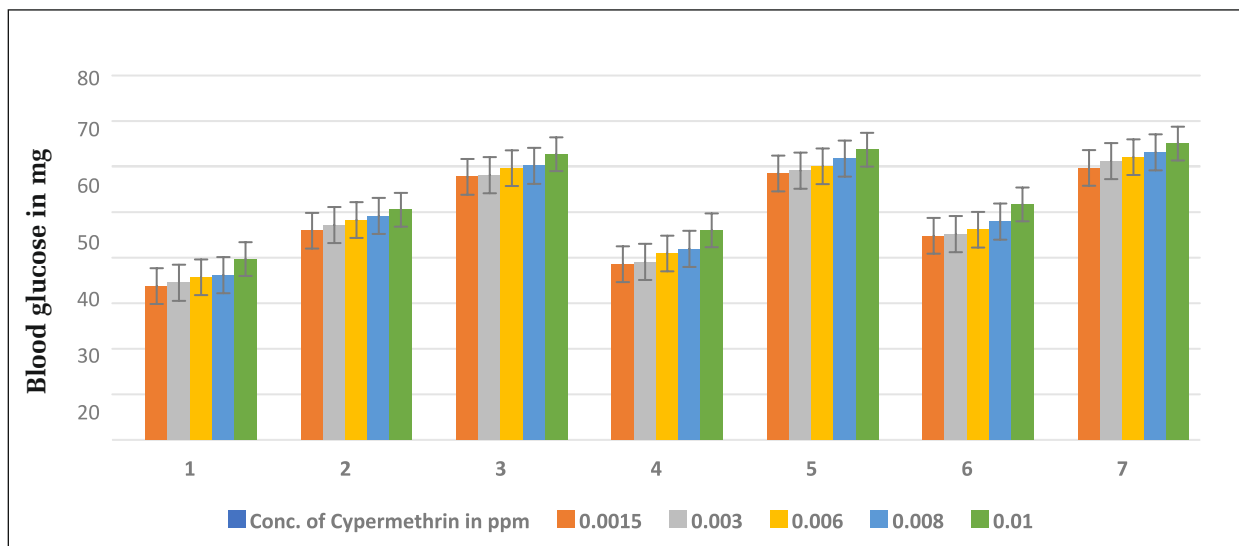


Fig 6: Effect of cypermethrin on the blood glucose in *Wallago attu*.

In the present investigation, *Wallago attu* exposed to different concentrations of cypermethrin shows an increase in blood glucose levels that were significantly ($P < 0.05$) increased without any mortality, demonstrating the response of exposed fish to metabolic stress. Stress stimuli trigger the fast release of these hormones from the fish's adrenal tissue, which is known to cause

hyperglycemia in animals. This rise could be attributed to stressed fish's increased gluconeogenesis response in order to meet their new energy demands (Winkaler *et al.*, 2007).

As the respiratory metabolism is depressed, stored intracellular glycogen is utilized under such conditions; the hyperglycemic hormone is

released for the degradation of glycogen and glucose thus leaked into the blood causing hyperglycemia (Hossain *et al.*, 2015).

CONCLUSION

The above findings clearly suggest that exposure of *Wallago attu* to cypermethrin, even at extremely low concentrations, was effective in altering the physiological processes of the fish, and that it can be recovered from pesticide effects by providing a healthy environment. In conclusion, assessing the physiological and biochemical impacts of pollutants in fish could be a useful method for predicting the subtle negative consequences of these materials on the aquatic ecosystem.

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