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Research Article

THERAPEUTIC EFFICACY OF *ALOE VERA* AGAINST THE EFFECT OF CYPERMETHRIN TOXICITY IN THE FRESH WATER FISH *CYPRINUS CARPIO*

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ABSTRACT

The pollution of environment due to use of pesticides has become an increasing problem over the last century with the development of industry, agriculture and increase in population. Heavy metals are extremely toxic and ubiquitous in natural environments and they occur in soil, surface water and plants, which readily mobilized by human activities such as mining and dumping of industrial waste in natural habitats such as forests, rivers, lakes and ocean Haematological parameters have been recognized as valuable tools for monitoring fish health. Haematological parameters were studied and compared different feeding behavior of teleost fishes, *Cyprinus carpio* were carried out in order to find out a normal range of blood parameters which would serve as baseline data for assessment of the health status of the fish as well as reference point for future comparative surveys. Blood parameters such as red blood cell count (RBC) and white blood cells count (WBC), haemoglobin, mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), mean cell haemoglobin, glucose, protein, cholesterol and urea were estimated from teleost fishes of different trophic level. Statistical analysis revealed that differences in haematological parameters between marine fish were significant. The result revealed that haematological RBC/WBC ratio; MCV and MCHC were significantly correlated study the impact of Lead acetate and ameliorative properties of *Aloe vera* is the freshwater fish *C. carpio*.

Keywords: Ameliorative properties, *Aloe vera, Cyprinus carpio*, hematology, Haemoglobin**,** Cypermethrin.

INTRODUCTION

The pollution of environment due to use of pesticides has become an increasing problem over the last century with the development of industry, agriculture and increase in population (Pugazhendy *et al.,* 2008). The organophos-phorous compounds are widely used because of their rapid biodegradability and non-persistent nature. Recent studies have proved that extremely low quantities of pesticides which enter the aquatic environment can affect productivity of organisms to kill eggs and larvae. The contaminations affect all group of organisms in aquatic ecosystem like invertebrate (Meenambal *et al*., 2012) non target aquatic biota like fish (Prashanth and Neelgund, 2007).

The actual number of pesticide related illnesses is unknown, since many poisonings go unreported. It has been estimated that at least three million cases of pesticide poisoning occur worldwide each year (Usha *et al.,* 2017a). The majority of these poisonings occur in developing countries where less protection against exposure is achieved. Knowledge of health risk and safety use is limited or even unknown. Studies in developed countries have demonstrated that the annual incidence of intoxication in agricultural workers can reach values up to 182 per million and 7.4 per million among full time workers (Calvert *et al*., 2004; Vasantharaja *et al.,* 2012).

Cypermethrin can be found in trace amount or at higher concentrations in soil and air. In mammals, cypermethrin can accumulate in body fat, skin, liver, kidney, adrenal glands, ovaries, lung, blood and heart (Wielgomas and Krechniak, 2007). Fishes are important sources of nation's diet, highly nutrients, easily digestible and its nutritional value depends on the biochemical

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composition (Padmapriya *et al*., 2017). Fish are extremely sensitive bioindicator of aquatic pollution and being preferred as a test species in toxicological screening of water (Usha *et al*., 2017b). Investigation on toxicity makes it possible to evaluate the effects of sublethal concentration on growth, behavior, physiology and biology of organisms, to determent their adaptation capabilities and to forecast possible consequences to toxic effect (Tamizhazhagan *et al*., 2017). Short term test is useful for routine monitoring for exploratory test and for, estimating effluent discharge. These tests determine LC_{50} which is a quick estimate of different toxicants and assessment of a toxicant to estimate toxicant concentration to be used in the intermediate and long term test. The intermediate test is conducted when a toxicity test is dealt with a long life cycle organism or longer life cycle stage which requires additional time for determination of LC_{50} (Mohamed and Gad, 2008).

Blood analysis is a valuable tool routinely applied for assessing the health and physiological status of animals (Borjesson *et al*., 2000). The fish blood exhibits changes in morphology of red blood cells when exposed to toxicants (Karuppasamy *et al.*, 2005). Haematological and biochemical profiles of blood can provide important information about the internal environment of the organism. Thus, the evaluation of haematological and biochemical characteristics in fish has become an important means of understanding normal and pathological processes and toxicological impacts (Kavitha *et al*., 2010). Typically, haematological parameters are nonspecific in their responses towards chemical stressors.

MATERIALS AND METHODS

Collection and Maintenance of the experimental animal

The freshwater fish *Cyprinus carpio* were collected from the fish farm located in Pinnalur village, near Vadalur, Cuddalore district. The fishes were brought to the laboratory and transferred to the rectangular fiber glass tanks (100 X 175 cm) of 500 liters capacity containing chlorine free aerated well water, fishes of the same size and weight were used irrespective of o their sex for the experiments.

Collection and preparation experiment plant

The dried *Aloe vera* powder was collected from Thookunampakkam Village near to Pondicherry. The *A. vera* powder was kept in carefully. *Cyprinus carpio* commonly called by ‘common carp (saatha kentai or thoppai kentai) is widely distributed in the freshwater on India. *C. carpio* was collected from the fish farm. The collected fish without the least disturbance were transported in polythene bags filled half with water. About 50 fish were put in each bag and water was well aerated, using pressurized air from a cylinder. This mode of transit proved successful since there was no mortality in all consignments through the course of this study.

Toxicity studies

Acute toxicity tests were conducted to measure the impact of toxicant on aquatic animals within a short period of five days. In the toxicity studies, the renewal technique of acute static test was adopted, in which fish were periodically exposed to the concentrations of the same composition, usually once in every 24 hrs by transferring the animals from one test chamber to another (Committee on methods for toxicity test with aquatic organisms, 1975).

Calculation of LC_{50}

The LC50 value was obtained by the probit analysis method based on the observed percentage of test animal’s serving at concentrations that were lethal to more than half and less than half of the test subjects (Finney, 1971). The experimental data were analyzed by taking the profit value of the percentages of mortality (Y), logarithm values of the administered dose multiplied 10000(X) and the average of both X and Y values (X and Y). The LC50 values were then calculated using the following formulae

Hematological Studies

Blood samples were collected from the control and experimental fish in the ductus cuvier with help of 24 gauge needle and stored in mechanized glass tube. The haematological parameters *viz.*, total red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), packed cell volume (PCV), mean cell haemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were determined by adopting the method of Pugazhendy *et al.* (2008).

Enumeration of red blood corpuscle

Blood samples were slowly sucked up by means of the Heamocytometer pipette till the mark 0.5 was reached (marked 0.5, 1.0 and 101). Then the diluting fluid was sucked as far as the mark 101. This produced a dilution of 1 in 200. While this was being done, the pipette was gently rotated so as to start the mixing. The pipette was firmly seized by its ends between the forefinger and thumb and shaken thoroughly for about one minute. The finger was then removed from the pipette and the diluted blood has been shaken out, a small drop was transferred to counting slide.

Enumeration of White Blood Corpuscel (WBC)

The total WBC count was made of haemocytometer’s Neubauer counting chamber. WBC was counted from the control and treated fish. The blood samples were drawn up to the 0.5 mark in WBC pipette and diluted upto the mark 11 with diluting fluid (Turk’s fluid = Gention violet, glacial acetic 3 mL and distilled water 7 mL). This produced a dilution o 1 to 20. The remaining producers were as the

same as above for the RBVC counting. For enumeration of leucocytes or sets of sixteen squares were counted out of nine squares. Instead of going over the squares in rows of four, wholes set of a sixteen could easily be counted at one time.

Estimation of haemoglobin (Hb) content

Haemoglobin content in blood was estimated using Sahli's Haemometer (Super, Germany) with permanent glass comparison standards and exposed in gm Hb/100 mL blood. Packed cell volume of blood was estimated by centrifuging blood in heparinized PCV tubes (Germany) at 7000 rpm/min for 30 min. the volume of blood taken and packed cell volume after centrifugation and packed cell volume per cent was calculated.

Mean Cell Volume (MCV)

The estimation of mean cell volume was completed from the values of packed cell volume and haematocrit percentage using the formula.

Mean Corpuscular Haemoglobin (MCH)

The mean corpuscular haemoglobin (MCH) content was computed from the values of haemoglobin content and erythrocyte count using formula expressed as pictograms.

Estimation of mean cell haemoglobin concentration (MCHC). Estimation of mean cell haemoglobin concentration (MCHC) was computed from the values of haemoglobin and the haematocrit percentage using the formula and expressed as percentage.

RESULTS

The quantitative fluctuation of haematological parameters like RBC, WBC, PCV, MCV, MCH, and MCHC of the freshwater fish *Cyprinus carpio* in all the groups are represent in table 1 and 2 and Figure 1. The following groups were as control (group 1), cypermethrin treated (group 2), cypermethrin along with *A.vera* exposure (group 3), *A. vera* (group 4), exposure to the sublethal concentration for the period of 24, 48, 72, 96 and 120 hrs respectively.

Table 1. Impact of cypermethrin on *Cyprinuscarpio* and sublethal concentration (LC_{50}) of 3 to 120 hours respectively.

Hours	LC_{50} concentration
3	**0.98**
6	0.93
12	0.86
24	0.70
48	0.67
72	0.56
96	0.58
120	0.50

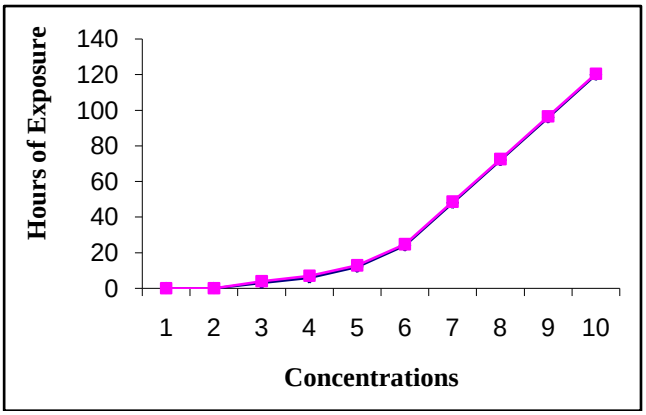

Figure 1. Graphical representation of LC_{50} concentration.

In the present investigation. The observed value of the WBC count of cypermethrin exposed fish (group 2) shows (Table 2) increased when compared to control fish (group 1). There are no noticeable changes in the control fish. Increased percent changes are 12,800, 7,000, 18,200, and 11,800 for 24 to 120 hrs respectively. While in the cypermethrin along with *A. vera* (group 3) treated fish, shows decreased treated in WBC content (gradually recovered against cypermethrin toxicity) when compared to group 2. The recovered percent changes are, 10.95, 11.40, 10.98, 9.83 and 13.51 for the period of 24 to 120 hrs respectively. While in fish exposed to *A. vera* alone (group 4) increased Hb content compared to control. The percent changes are 12.4, 11.0, 13.4, and 11.6 for the period of 24 to 120 hrs respectively. The observed Hb content in blood cell counts for group 2, 3 and 4 is statistically significant at 1% and 5% levels.

PCV levels in cypermethrin exposed group 2, when compared with control (group 1). The percent changes are 48.8, 35.0, 51.2 and 48.9 for 24 to 120 hrs respectively. Group 3 cypermethrin along with *A. vera* exposure, the PCV levels are increased (regained), when compared to group 2. The overall percent changes are, -3.47, -3.70, -3.44, -4.11, and 5.09 for 24 to 120 hrs respectively. Group 4 *A. vera* supplemented fish, the PCV level was decreased when compared to group 2, 3 and nearly too normal in control. The percent changes are 15.06, -8.72, 16.40, 17.39, and 19.51 for the period of 24 to 120 hrs respectively. PCV content in blood cell count of groups 2, 3 and 4 is statistically significant at 1% and 5% levels.

The cypermethrin exposed fish (group 2) shows a slight decrease in the MCV content when compared to control fish. The percent changes are -62.2, 61.6, 64.5 and 62.4 24 to 120 hrs respectively. Group 3 cypermethrin along with *Spiruina* exposure fish, the recorded MCV content is significant regained against the cypermethrin toxicity. The overall recovered percent changes are, -5.95, -7.36, -7.70, -2.56 and -8.04 for the period of 24 to 120 hrs respectively. Group 4 *A. vera* supplemented fed exposed to fish, the MCV levels are increased, when compared with all other 3 groups. The overall increased percent changes are

9.01, 4.29, 5.79, 4.86, and 7.10 for the period of 24 to 120 hrs respectively. In the present investigation, the MCV content in blood cell counts for groups 2, 3 and 4 is statistically significant at 1% and 5% levels.

The observed values of MCH are decreased in cypermethrin exposure (group 2) when compared with control (group 1). Decreased percent changes are 14.0, 16.6, 17.1 and 16.1 for 24 to 120 hrs respectively. Cypermethrin along with *A. vera* exposure (group 3) shows increased in MCH content, when compared to group 1, and 2. Increased percent changes are -3.66, -7.21, -4.94, -3.29 and -5.29 for the 24 to 120 hrs respectively. The *A. vera* supplemented exposure fish, the level of MCH in blood content gradually increased, when compared with all other 3 groups. Increased percent changes are 6.80, 4.10, 4.09, 3.37, and 3.49 for 24 to 120 hrs respectively. The observed MCH content in blood counts for groups 2, 3 and 4 is statistically significant at 1% and 5% levels.

The level of MCHC content increased to cypermethrin exposure, (group 2) when compared with control (group 1). Increased percent changes are 17.64, 17.44, 19.44, 14.29, and 22.06 for the period of 24 to 120 hrs respectively. The cypermethrin along with *A. vera* exposure to fish, the MCHC content levels are decreased when compared to group 2. The percent changes are 7.47, 6.35, 7.04, 7.13, and 4.46, for the period of 24 to 120 hrs respectively. The *A. vera* supplemented fed exposure (group 4) MCHC values are decreased, when compared with group 2 and 3, which is near to control. The percent changes are 26.0, 25.0, 23.6 and 22.1 for the period of 24 to 120 hrs respectively. The increased and decreased level of MCHC content in blood of all groups is statistically significant at 1% and 5% levels.

Table 2 Therapeutic effect of *Aloe vera* against Cypermethrin induced toxicity in the blood parameters of *Cyprinus carpio.*

Experimental group	RBC (cu/mm)	WBC (cu/mm)	Hb g (%)	PCV (%)	MCV (μm^3)	MCH (pg)	MCHC (%)
Control	6.9±0.41	12,800±14.0	12.4±0.62	48.8±2.52	62.2±3.40	14.0±1.02	26.0±1.70
Cypermethrin	4.68±0.32* – 28.10	7,000±10.4** –45.3	11.0±0.60* –25.37	35.0±1.42* –27.7	61.6±3.15** –0.93	16.6±1.45** –2.22	25.0±1.42** –3.22
Cypermethrin + *Aloe vera*	6.97±0.43** + 0.88	18,200±11.46** + 42.18	13.4±0.52** + 7.46	51.2±2.35** + 4.8	64.5±3.10** + 2.02	17.1±0.85* + 0.55	23.6±1.44** + 2.22
Aloe vera	6.97±0.44** + 0.88	11,800±12.13* + 0.46	11.6±0.50** + 1.49	48.9±2.44** + 0.20	62.4±3.10** + 0.31	16.1±0.82** + 0.05	22.1±1.68** + 0.71

(+/–) Indicate the percentage change over control. Mean ± SE (mean of five individual observations). *Significant at 1% level and **Significant at 5% level.

DISCUSSION

The present investigation showed that, the RBC, haemoglobin and haematocrit values are significantly decreased in cypermethrin treated fish when compared to control. The decreases in RBC might be due to the effect of pesticides on blood forming organs (Bone marrow and liver) and inhibition of many steps of biosynthesis of fish, as the results of pesticide exposure. But the exposure of *A. vera* supplements diet, which is having highly rich nutrients so, gradually increased in RBC in group III and IV, when compared with control.

The decreased value of content indicates acute anaemia. The anaemia could be due to destruction of RBC. Similarly, the erythrocyte number of another fish was exposed to cypermethrin for a period of 24, 48, 72, 96 and 120 (Tort and Torres, 1988). In the erythrocytes of trout apoptotic reaction was observed when it was exposed to the tributylin toxicity (Tiano, 1995). In the present study, it is found that a reduced RBC contents in the cypermethrin exposed fish, may be due to inhibition of erythrocyte production or decrease in the rate of erythrocyte destruction. Goel and Kalpana (1985) have reported that the RBC count and haemoglobin content values significantly decrease resulting macrocytic anaemia in *Heteropneustes fossilis* exposed to zinc. According to Hussein *et al.* (2008), *Oreochromis niloticus* and *Chrysichthyes auratus* which exposed to cypermethrin result in significant decrease in RBC, haemoglobin. It may be attributed to the lowering of the oxygen content of the water (Usha *et al.*, 2017)

Fresh water fish *C. carpio* was exposed to cypermethrin resulted in the erythrocyte system dysfunction, as evidence by haemolytic anaemia observed on the onset of the experiment and related to rapid erythrocyte disintegration (Thomas *et al.*, 1999, Tamizhazhagan, 2015). It was observed that the structure of red blood cells of fish exposed to toxic elements and environmental pollution had deformed and the erythrocytes decreased in erythrocyte number (Pacheco and Santos, 2002). Due to toxicity, the morphology of blood cells changed and generally elongated horizontally (Jeney *et al.*, 1997; Comelekoglu *et al.* (2000).

RBC is important when investigating anaemia transport and excretion of nutrients, oxygen, body wastes and carbonic acid gas (Kim *et al.*, 2005). Erythrocyte level was found to be depressed in fish subjected to a stressful

condition. Changes in the erythrocyte profile suggest a composition of oxygen deficit in the body due to gill damage and the nature of the changes shows a release of erythrocytes form they blood deposits.

In the present study, the significant decrease in RBC and haemoglobin content might have resulted from the lowering of the oxygen content of the water due to the presence of cypermethrin in the test media. The increase in WBCs content recorded in present work when compared with control and other parameters could be due to the attempt of the fish to fight against the antigens (toxicants) and this augmented the production of more WBC to improve the health status of the fishes which agreed with the reports of Adeyemo (2005) and Gabriel *et al.,* (2007). However, the total Leucocyte Count (TLC) was lower in control fish compared to those exposed to the toxicant. The increased in TLC in the exposed fish was dose-dependent. Changes in leukocyte count could be due immunological reactions to produce antibodies to cope up with stress induced by toxicants (Yaji and Auta, 2007). The increased in WBC count can be correlated with an increased in antibody production which helps in survival and recovery of fish exposed to sub-lethal concentration of pesticides (Tamizhazhagan and Pugazhendy, 2016)

The increase Hb content could be explained as a process where the body tires to replace the oxidized denatured Hb (Cyriac *et al.,* 1989). The experimental values of PCV (Packed Cell Volume) in the fish exposed to cypermethrin, when compared to control PCV content were decreased. The recorded values of Group III Cypermethrin and *A. vera* exposed to fish, values were decreased. Group IV *A. vera* alone exposed to fishing shows increases in the PCV content. Haematocrit values of *C. carpio,* which was exposed to different cadmium concentration, showed differences (Venkatesan *et al.,* 2012). The fish exposed to cypermethrin were decreased in the packed cell volume levels and fall in the number of red blood cells followed by PCV confirms anaemia in *Clarias batrachus.*

The result shows Erythrocytes and inhibition of erythropoiesis, which are confirmed by increased in MCH values. The cypermethrin exposed fish showed a reduction in the mean corpuscular haemoglobin. The reduction in the erythrocyte count and the haemoglobin content accompanied by increased MCH and MCV was revealed (Meenambal and Pugazhendy, 2012) in carp exposed for 24 hours to cypermethrin. The observed values MCHC in group II cypermethrin treated fish, when compared to control. Erythrocyte and inhibition of erythropoiesis, which is confirmed by increased MCHC values. The MCHC levels increased in tilapia *Oreochromis mosambicus* when it was exposed cadmium (Ruperalia *et al.,* 1990), our results agree with those obtained in previous studies. Some authors reported by cadmium exposure resulted in increased in MCHC.

CONCLUSION

The present study impact of cypermethrin and therapeutic efficacy of *Aloe vera* treated fish's aquatic ecosystems can affect aquatic fauna in different ways. Alterations in physico-chemical properties of water, destruction of the delicate balance of the environment, entry into the food chains and physiological damage to the vital tissues of aquatic fauna are the threatening issues of the modern day pesticides. Long term exposure to these products causes countless abnormalities and reduces the life span of aquatic organisms. Blood biochemical alteration occurs and many changes fish body .Finally, we conclude that cypermethrin is highly toxic to fish, and impose life threatening effect on fish at both lethal and sublethal concentrations. Altered haematological responses can be used as tools in bioassessment to monitor ecotoxicological risks associated with pesticides such as cypermethrin to various fish. It affected entire aquatic food chains to signals researchers to provide awareness common mans.

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