



AMELIORATIVE EFFECT OF *SOLANUM VIRGINIANUM* (Lin) AGAINST LEAD ACETATE TOXICITY IN THE FRESH WATER FISH *CYPRINUS CARPIO* ON HEMATOLOGICAL ALTERATION

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ABSTRACT

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 with the impact of Lead acetate and ameliorative properties of *Solanum virginianum* on the freshwater fish *C. carpio*.

Keywords: Ameliorative properties, *Solanum virginianum*, *Cyprinus carpio*, Hematology, Haemoglobin.

INTRODUCTION

The increase in population, increased human activities, indiscriminate use of natural resources and dumping of wastes cause water pollution (Jayachandran, and Pugazhendy, 2008). Pollution of aquatic ecosystem is posing a great challenge due to regular mixing of different industrial effluents agrochemicals like fertilizers, insecticides, detergents, pesticides and plant toxin in water (Meenambal *et al.*, 2012). Water pollution is recognized globally as a potential threat to both human and other animal populations which interact with the aquatic environment (Svensson *et al.*, 2004). Environmental pollution is one of the most serious problem facing human beings and all other living things, today with the rapid growth in industrialization, population explosion and technological advancement, man's activities have

tremendously affected the environment (Sharma *et al.*, 2002).

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 (Larison *et al.*, 2000). As a result, heavy metals pose a potential threat to terrestrial biota. They are known to cause profound reproductive loss in animals (Eeva *et al.*, 1997). The weathering of rocks, soil forms and increased use of metal containing fertilizers in agriculture could lead acetate to a continued rise of the concentration of metal pollutants in freshwater reservoirs as a result of water runoff, thereby representing the greatest hazard to human consumers of fish (Marr and Creaser, 1983; Gutenmann *et al.*, 1988).

Heavy metal constitutes a serious type of pollution in fresh water and being stable compounds. They are not readily removed by oxidation, precipitation or other processes and affect the activity in the recipient animal (Nammaluwar, 1985). Heavy metals enter into aquatic habitats by a number of routes and cause hazardous effect on their morphology and physiology. This may lead acetate to the destruction of beneficial species either indirectly through breaking the biological food chain or directly by affecting the aquatic forms of life. Heavy metals such as chromium, mercury, lead acetate and arsenic are not essential elements and are toxic to aquatic organisms even at low levels. Among the heavy metals lead acetate (Pb) are found at relatively high levels in crops, vegetables and pasturage herbs for animals (Chen *et al.*, 1999; Cheng and Hardy, 2003). These metals are transported and concentrated through the food chain and can accumulate in the human body and cause chronic poisoning, lead acetate to cancers and other diseases (Cheng, 2003).

Lead acetate is one of the oldest known metals and also one of the most widespread toxicants, which poisoning remains a health threat (Hernberg, 2000). Lead architectonic is probably the most common form of heavy metal intoxication. It is well documented that, one of the most dangerous and insidious poisons. It continuous environmental and occupational exposure may contribute to renal, nervous, hepatic, hematological and reproductive disorders in man and animals (Flora *et al.*, 2006; El-Sayed and El-Neweshy, 2009). Lead acetate is ubiquitous in the environment as a result of its natural occurrence and its industrial use. The primary sources of lead acetate are lead acetate paints acidic foods and beverages, including tomato juice, fruit juice, cola drinks, cider and pickles can dissolve the lead acetate when packaged or stored in improper glass containers. Food and beverages thus contaminated cause lead acetate poisoning (Trivedi and Gurdeep Raj, 1992). Currently lead acetate exposure usually results from lead acetate in deteriorating household paints in the work place, crystals and ceramic containers that leaches into water and food, lead acetate use in hobbies and use in some traditional medicines and cosmetics. Lead a is naturally occurring element and a systemic toxicant that affects several organs in the body including the kidney, liver, central nervous system, haematopoietic system, endocrine system and reproductive system. Exposure during early stages of human life poses a risk for the health and functional abilities of vulnerable fetus and infants. Concerns exist that the possibility of developmental neurological exposure may result in an acceleration of age-related CNS decline in function. Lead acetate poisoning causes renal dysfunction, liver cirrhosis, damage to the central nervous system and anemia (Sheffield *et al.*, 2001; Damek *et al.*, 2012). Acute and chronic lead acetate exposure can seriously affect human health. It is a confirmed multi-target toxicant with effects in the gastrointestinal, haematopoietic, cardiovascular, nervous immune, reproductive and excretory system (Daggett *et al.*, 1998; Bonde *et al.*, 2002).

In humans and experimental animals significant accumulation of lead acetate in the blood and tissues occur following environmental exposure (Areola *et al.*, 1999). Lead acetate poisoning is the most important environmental health problem (Committee on Environmental Health, 1993). Children may appear inattentive, hyperactive and irritable even at low lead acetate exposure. Children with greater lead acetate levels may be affected with delayed growth, decreased intelligence, Short-term memory and hearing loss. At higher levels, lead acetate can cause permanent brain damage and even death (Cleveland *et al.*, 1999).

Toxic effects of lead acetate also include nephrotoxicity (Nolan and Shaikh, 1992), hepatotoxicity and cardiovascular damage (Gajawat *et al.*, 2005). The carcinogenic effect of lead acetate has been receiving increasing attention (IARC, 1993; Silbergeld *et al.*, 2000). Lead acetate is classically a chronic or cumulative toxic. Acute effects including gastrointestinal disturbances (anorexia, nausea, vomiting and abdominal pain); neurological effects (encephalopathy, malaise and drowsiness); hepatic and renal damage and hypertension have been reported by Flora (2006). Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological changes in fishery management and disease investigation (Jayachandran, and Pugazhendy, 2008). Haematological abnormalities under toxic stress may also be reflected in other physiological activities like oxygen consumption and metabolism which result in death (Meenambal *et al.*, 2012). Analysis of haematological parameters can be beneficial in assessing fish health (Silbergeld *et al.*, 2000) but the variation in fish hematologic parameters has been a concern for researchers and health specialists. Free amino acids would also serve as precursors for energy production under stress and the synthesis of the required proteins to face the stress. The assessment of protein and total amino acid content can be considered as a diagnostic tool to determine the physiological phase of the cell (Usha *et al.*, 2017).

Studies carried out on fish have shown that heavy metal may have toxic effects, altering physiological activities and biochemical parameters both in tissues and in blood (Bonde *et al.*, 2002). Enzyme assays can make important contributions to the diagnosis of disease because very small changes in the concentration of an enzyme activity can be easily measured (Foster, 1980). Dehydrogenase is the enzyme involved in the energy release of the biological oxidation of food stuff inside the mitochondria and also in the production of reduced potential (NADPH) required in the biosynthetic and detoxification mechanisms. Lactate Dehydrogenase (LDH) is a pivotal enzyme between the glycolytic pathway and tricarboxylic acid cycle and any changes in protein and carbohydrate metabolism might cause changes in the LDH activity (Sheffield *et al.*, 2004). Lead acetate directly affects the hematopoietic system through restraining the synthesis of hemoglobin by inhibiting various key enzymes involved in the heme synthesis pathway. It also reduces the

life span of circulating erythrocytes by increasing the fragility of cell membranes. The combined aftermath of these two processes lead acetates to anemia. Anemia caused on account of lead acetate poisoning can be of two types: hemolytic anemia, which is associated with acute high-level lead acetate exposure, and frank anemia, which is caused only when the blood lead acetanilid is significantly elevated for prolonged periods. It has been reported as a major mechanism of lead acetaldehyde toxicity. Under the influence of lead acetate on set of oxidative stress occurs on account of two different pathways operative simultaneously; first comes the generation of ROS, like hydroperoxides (HO₂), singlet oxygen and hydrogen peroxide (H₂O₂), and second, the antioxidant reserves become depleted (Padmapriya *et al.*, 2017). The present study was aimed to investigate the ameliorative effect of *Solanum virginianum* against lead acetate toxicity in the fresh water fish *Cyprinus carpio* on hematological alteration.

MATERIALS AND METHODS

Collection and maintenance of the experimental animals

The freshwater fish *C. carpio* obtained from the Navarathna fish farm nearby Pinnaloor, Cuddalore district. Fishes were safely brought to the laboratory and transferred to the rectangular cement tanks (100 × 175 cm) of 500 liters capacity containing chlorine free well water, fishes of the same size and weight were used irrespective of their sex for the experiments.

Procurement and rearing of the experimental fishes

C. carpio common carp commonly called rohu is widely distributed in the freshwater of 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 well aerated, using pressurized air from a cylinder. This mode of transit proved successful since there was no mortality in all consignments throughout the course of this study. Selection of Plant: Whole plants of *S. virginianum* were cleaned and chopped into small pieces and dried under shade. The coarse powder was obtained by mechanical grinding. The powdered material (100 g) was subjected to continuous hot extraction in soxhlet apparatus at a temperature of (60-700°C) by using ethanol (95% v/v) as solvent. After complete extraction, the extract was dried. The yield was about 5% w/w and it was stored at 4°C in desiccators.

Haematological Studies

Collection of blood

Blood samples were collected from the control and experimental fish in the ductus Cuvier with the help of 4 gauge needle and stored in heparinized glass tube. The hematological parameters *viz.* total Red blood cells (RBC),

White blood cells (WBC), Haemoglobin (Hb), Packed cell volume (PCV), Mean cell haemoglobin (MCH), and Mean cell hemoglobin concentration (MCHC) were determined by adopting the method of Daecie and Lewis (1984)

Enumeration of red blood corpuscles (RBC)

Blood samples were slowly sucked up by mean of the Haemocytometer pipette till the mark 0.5 was reached, 1.0 and 101). Then the diluting fluid was sucked as far as the mark 101. This produced a dilution of 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 diluting fluid in the capillary tube blown out. After a few drops of the diluted blood had been shaken out, a small drop was transferred to the counting slide.

Enumeration of white blood corpuscles (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 acid 3 mL and distilled water 97 mL). This produced a dilution of 1 in 20. The remaining procedures were as the same as above for the RBC counting. For enumeration of leucocytes four sets of sixteen squares were counted out of nine squares. Instead of going over the squares in rows of four, whole set of a sixteen could easily be counted at one time.

Estimation of haemoglobin (Hb) content

Haemoglobin content in the blood was estimated using Sahli's Haemometer (Superior, Germany) with permanent glass comparison standards and exposed in gm Hb /100 mL blood.

Determination of packed cell volume (PCV)

Packed cell volume of blood was estimated by centrifuging blood in heparinized PVC 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 the 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

Haematological Parameters

The quantitative changes of haematological parameters like RBC, WBC, Hb, MCV, MCH, MCHC, PCV, and Serum SGOT, SGPT of the blood cells in the fresh water fish *C. carpio* in all groups control (group 1), sublethal concentration of lead acetate (group 2), lead acetate along with *S. virginianum* (group 3) and *S. virginianum* supplementary feed alone (group 4) exposed to 24, 48, 72, 96 and 120 hours are observed.

Red blood corpuscles (RBC)

The chelation effect of *S. virginianum* of the toxic impact of lead acetate on *C. carpio* blood parameters is shown in Table 1. Lead acetate administration fish, the RBC number was decreased significantly, the percent decreased from RBC over the control fish was -28.10, the combination of *S. virginianum* with lead acetate treated fish group III shows the RBC content just recovered when compared to lead acetate treated group II. The percent changes over control were 0.886 while in the *S. virginianum* alone exposed group IV recorded not many changes in the RBC content. The statistical analysis in between the four various groups were highly significant at 5% and 1% level.

White blood corpuscles (WBC)

In the blood of lead acetate treated fish group II the numbers of white blood cells (WBC) were significantly decreased, the percent decrease was -45.3. When compared to control group I, where as in the combined exposure of lead acetate acetate + *S. virginianum*, the WBC content was slowly recovered. The percent recover was 42.18 compared to the group II. The *S. virginianum* treated (supplementary) group IV. WBC content was not much affected the slight variation was noticed. The percent changes were 0.46 when compared to control. Statistical analysis shows between the groups were highly significant at 5% level (Table 1).

Haemoglobin (Hb)

Haemoglobin concentrations were varied during the period of study. The fish treated with lead acetate the haemoglobin content were decreased when compared to control, the percent decrease was -25.37 where as in the lead acetate acetate along with *S. virginianum* the haemoglobin content were significantly recovered, the percent increase the Hb in

the group III was 7.46 and the group IV, *S. virginianum* alone supplemented group fish shows that not much variation recorded. The percent changes were 1.49 when compared to the control fish at 120 hours treatment. The recorded Hb content in 4 different groups was significant at 5% and 1% level (Table 1)

Packed cell volume (PCV)

In the blood of control fish, the mean number of packed cell volume was 49.8 ± 2.52 . The decreased number of packed cell volume was recorded in lead acetate treated group II (-27.7) whereas the fish treated with lead acetate along with *S. virginianum* group III. Shows the recovery from the effect of lead acetate treated fish group II. The number of packed cell volume was significantly decreased (4.8) when compared to lead acetate treated group II and the *S. virginianum* alone supplementary group IV shows there is no effect in this group. Statistical analysis recovered the significantly between groups at 5% and 1% level (Table 1)

Mean cell volume (MCV)

The MCV of control and lead acetate treated fish was represented in Table 1 The means number of MCV was 64.2 ± 3.40 . The percent decrease of MCV was -0.93 in the lead acetate treated group II. While in lead acetate along with *S. virginianum* treated group III shows recovery of fish from the lead acetate treated group II. The percent recovery was 2.02. Whereas the *S. virginianum* alone treated group IV shows without any change in this group fish. The recorded values in this group were significant at 5% and 1% level.

Mean corpuscular haemoglobin (MCH)

The control and treated groups of fish the MCH content was represented in Table 1 the mean corpuscular haemoglobin in the control group I was 18.0 ± 1.02 . Lead acetate treated group II shows the gradual decreased MCH when compared to control group I. The percent decrease value of MCH was -2.22. The lead acetate along with *S. virginianum* treated group III showed a gradual recovery from the lead acetate acetate treated group II. The percent recovery was 0.55 whereas in group IV in *S. virginianum* alone treated fish showed without much effect. The numbers of mean corpuscular haemoglobin recorded were significant at 5% and 1% level.

Mean cell haemoglobin concentration (MCHC)

The MCHC value of control and lead acetate treated groups was represented in Table 1 The mean cell haemoglobin concentration in lead acetate treated fish showed significantly increased the mean value of lead acetate treated group (-3.22) when compared to control group I. Fish treated with lead acetate along with *S. virginianum* group III showed the recovery from the lead acetate effect. While in the group IV, *S. virginianum* alone treated fish showed without any change in the MCHC value.

Table 1. Chelating effect of *Solanatum virginianum* against lead acetate induced toxicity in the blood parameters of *Cyprinus carpio*.

Experimental group	RBC (cu/mm)	WBC (cu/mm)	Hb g (%)	PCV (%)	M CV (μm^3)	MCH (pg)	MCHC (%)
Control	7.9±0.41	12,800±14.0	13.4±0.62	49.8±2.52	64.2±3.40	18.0±1.02	27.0±1.70
Lead acetate	5.68±0.32*	7,000±10.4**	10.0±0.60*	36.0±1.42*	63.6±3.15**	17.6±1.45**	27.0±1.42**
Lead acetate	-28.10	-45.3	-25.37	-27.7	-0.93	-2.22	-3.22
Lead acetate + <i>Solanatum virginianum</i>	7.97±0.43** + 0.88	18,200±11.46** + 42.18	14.4±0.52** + 7.46	52.2±2.35** + 4.8	65.5±3.10** + 2.02	18.1±0.85* + 0.55	27.6±1.44** + 2.22
<i>Solanatum virginianum</i>	7.97±0.44** + 0.88	11,800±12.13* + 0.46	12.6±0.50** + 1.49	49.9±2.44** + 0.20	64.4±3.10** + 0.31	18.1±0.82** + 0.05	28.1±1.68** + 0.71

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

DISCUSSION

In the present investigation *C. carpio* fish exposed to sublethal concentration of lead acetate and supplementary feed *S. virginianum* recovered shows a significant changes of haematological parameters like RBC, WBC, haemoglobin, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), packed cell volume (PCV) (Svensson *et al.*, 2004). Haematological parameters can provide information on nutrient status, digestive function and routine metabolic level of fish may be confronted with stress factors such as varied water quality, pollution and disease; fish can adapt themselves to bad environmental conditions by changing their physiological activities. A number of hematological indices such as RBC, WBC, Hb, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV), packed cell volume (PCV) are used to assess the functional status of the blood stream and have been used as an indicator of pollution in aquatic environment. Haematological and clinical chemistry analyses are not used extensively owing to the lack of standard values for various fish species (Tamizhazhagan, 2015; Larison *et al.*, 2000).

In the present investigation shows a decreased level of RBC count. Hb concentration PCV and MCHC, protein, albumin and globulin of blood in lead treated fish and increased MCV MCH values. The reduction of RBC count and Hb content may be due to the destructive action of lead on erythropoietic tissue as a result of which the viability of the cell might have been affected. Decrease in RBC count, Hb content and PCV were symptoms of fish suffer anemia (Eeva *et al.*, 1997). In addition increase in MCV, MCH and decreased MCHC values indicate that the anemia was of a macrocytic type (Gutenmann, 1998). Similar result was observed in acute effect of diazinon on carp (Tamizhazhagan and Pugazhendy, 2016). Swelling of RBC due to hypoxic condition in the toxicant treated organisms

may lead to a significant increase in MCV values as suggested by Wepener *et al.* (1992). The increase in MCV may also result from an increase of immature RBC (Carvalho and Fernandes, 2006). A Reduction of haematological values indicated anemia in the pesticide exposed fish. This may be due to erythropoiesis, hemosynthesis and osmoregulatory dysfunction or due to an increase in the rate of erythrocyte destruction in hematopoietic organs.

Saxena and Seth (2002) have observed significant changes in the hematology of the common fresh water fish *Channa punctatus* on lead exposure. Parma *et al.* (2007) has been documented lead alter the hematological parameters in *Prochilodus lineatus*. They also suggest changes in haematological parameters might have been brought about by lead as an anemic condition due to decrease synthesis of Hb and RBC number in bone marrow cells. Goel and Kalpana (1985) have reported that the RBC count and haemoglobin content values significantly decrease resulting microcytic anemia in *H. fossilis* are exposed to zinc. Lead can have two modes of action on blood cells. It may either induce oxidative stress. As a hydrophobic compound it may accumulate in cell membranes and disturb membrane structure (Michelangeli *et al.*, 1990). Reduction of Hb content could be due to either an increase in rate at which Hb is destroyed or a decreased in the rate of Hb synthesis (Moss *et al.*, 1964). Decreased Hb content may be a consequence of changes in the number of circulating erythrocytes. Similar findings were reported in goats (Khan *et al.*, 2009) and rabbits (Yousef *et al.*, 2009).

In the present investigation lead treated fish (group 2). WBC count has increased and recovery group remarkable increase in WBC. WBC as key components of innate immune defense and leukocytes are involved in the immunological function in the organisms (Chen *et al.*, 1999) has been reported increasing the WBC count during sublethal treatment of arsenic on *Catla catla*, Increasing

WBC might be resulted from stimulation of the immune system and to protect the fish against toxicity. Jayachanadran and Pugazhendy (2008), have reported the increase of WBC and decrease of haematotoxic in the teleost fish *C. carpio* exposed to neem based pesticide. Increase in the total leukocyte count has been attributed to several factors like increase in thrombocytes, lymphocyte or squeezing of WBC in peripheral blood (Padmapriya *et al.*, 2017).

CONCLUSION

The present study impact of lead acetate and ameliorative properties of *S. virginianum* treated fishes aquatic ecosystems can affect aquatic fauna in different ways. 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 lead acetate 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 lead acetate to various fish. It affected entire aquatic food chains spread generation to another generation.

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