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Removal of cadmium from freshwater fish common carpio *Cyprinus carpio* using *Abutilon indicum* Leave powder

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Abstract
Present study deals with the investigation of *Abutilon Indicum* activity on the haematological limits in the blood of Common carpio (*Cyprinus carpio*). In the present study, the Red blood cells (RBC), White blood cells (WBC) and Haemoglobin (Hb) was observed in the blood of Coomon carpio (*Cyprinus carpio*) treated with *Abutilon indicum* extract and the result showed that the RBC counts, WBC counts, Hb, were much increased when compared to the control. 0, 2, 4, 6 and 8th day of RBC, WBC, and Hg levels were <0.05> significantly elevated in the experimental fish over the control and the WBC level was decreased significantly <0.05> in experimental fish.

Keywords: *Cyprinus carpio, Abutilon Indicum*, haematology

Introduction
Fishes are an important part of human nutrition, and those from the contaminated sites showed a potential risk to human health. Since, fish occupy the top of the aquatic food chain, they are suitable indicators of metal contamination. Metals are well-known inducer of oxidative stress, and assessment of oxidative damage and antioxidant defences in fish can say metal contamination of the aquatic environment (Livingstone 2003) [1]. Aquatic organisms are in direct content with the environmental and are susceptible to any changes that may occur. The introduction of many of the relatively toxic heavy metal captions in small amounts into aquatic environmental causes multiple changes in the internal dynamic of aquatic organisms, even at sub lethal levels. By measuring specific physiological and biochemical alterations in the blood of fish exposed to short periods of sub lethal stressors may provide a sensitive methods for predicting the effect of chronic exposure and survival reproduction and growth (Mckee and Walf 1963) [2]. The most nonessential heavy metals of particular concern to fish and surface water are cadmium (Cd), Lead (Pb) and mercury (Hg) which enter into fish mainly via gills. The progressive and irreversible accumulation of these metals in various organs of marine creatures ultimately leads to metal related diseases in the long run because of their toxicity, thereby endangering the aquatic biota and other organisms including humans. Zinc is an essential trace metal, becomes toxic when the nutritional supply becomes excessive. The main uses of zinc are in the manufacture of galvanized iron, bronze, paint (white), rubber, glazes, enamel glass, paper, as a wood preservative (ZnCl2, fungicidal action), petrochemicals, and fertilizers and in steam generation power plants etc. (Nriagu and Pacyna 1988) [3]. Some zinc is released into the environment by natural processes, but most comes from activities of people like mining, steel production, coal burning, and burning of waste. It attaches to soil, sediments, and dust particles in the air. Zinc compounds can move into the groundwater and into lakes, streams, and rivers. Most of the zinc in soil stays bound to soil particles. Moderately increased zinc concentrations in water stemming from the release of zinc from drainage pipes due to corrosion. It builds up in fish and other organisms (Joshi 1990) [5]. Studies carried out on various fishes have shown that heavy metals may alter the physiological activities and biochemical parameters both in tissues and in blood. The toxic effects of heavy metals have been reviewed, including bioaccumulation. The organisms developed a defense mechanism against the deleterious effects of essential and non-essential heavy metals and other xenobiotics that produce degenerative changes like oxidative stress in the body (Sahan *et al*. 2007) Heavy metal pollutants cause massive fish kill and destruction of other aquatic life

(Malik *et al*. 2010) [6]. Fish in close association with their aquatic environment and any changes in this environment would be reflected in alterations in their haematological studies (Rashed 2001) [7]. Stresses and pollutants generally cause rapid changes in blood characteristics of fish (Shah 2002) [8]. Copper and Lead are stable and persist environmental pollutants and are considered as strong toxic metals to aquatic organism. These pollutants cause serious effects on growth, physiology and survival rate of aquatic organism especially fish16. With increasing emphasis on pisciculture and greater awareness of the pollution of natural water resources, haematological studies in fish have assumed greater significance. Zinc toxicity depends on pH, which controls the concentration of zinc in solution. High concentrations of zinc can cause toxicity in plants. Hence, the present study has been carried out the Removal of cadmium from freshwater fish Common carpio *Cyprinus carpio* using *Abutilon indicum* Leave Powder.

Materials and Methods

A live fish (12± 1g) were collected from High-tech fish farm, Madurai, Tamil Nadu, India. The fishes were maintained in non-chlorinated water in 20 day.

The ground nut oil cake, fish meal and rice bran, tapioca, soybean, were mixed and sterilized and mixed to a multivitamin tablet and different concentrations 0.2ppm, 0.3ppm and 0.4ppm) of *Abutilon indicum* extract used for experimental fishes and without plant extract diet for control fish. The food was made into small pellets. In every eight in days following haematological studies such as, (Table 1).

Haematology

Hemoglobin content of the blood was estimated by Cyanmethemoglobin method (Drabkin, 1946).

Haemoglobin content

Shali's acid haematin method

Principle

Hemoglobin can undergo several reactions; it binds with oxygen and carbon monoxide to from oxy hemoglobin and carboxy hemoglobin, respectively. Oxidation of the fesrrous ion to the ferric form results in the formation of methemoglobin. Methemoglobin binds cyanide ions to form cyanmethaemoglobin. Hemoglobin can be measured in any of these forms, but the most satisfactory method of assay from the view point of accuracy and simplicity involves the conversion of all forms of blood hemoglobin to cyanmethaemoglobin.

Hemoglobin + K3 Fe (CN) 6 ⟶ Methemoglobin

Methemoglobin + KCN ⟶ Cyanmethemoglobin the brownish colored cyanmethemoglobin is the product of almost all form of hemoglobin found in blood except HBS and can be measured at 540 nm. The colour intensity at this wavelength is proportional to the total hemoglobin concentration.

Reagents

Reagents 1: Hemoglobin reagent

Reagents 2: Hemoglobin standard (15g/dl)

Procedure

Fill the graduated tube to the 20 mark (on % scale) with 0.1N HCl. Draw blood by using haemoglobin pipette to 0.02ml. Wipe the tip of the pipette with cotton, so that no blood is left to stick to its outside. Expel blood into the Shali tube containing the HCl solution. Suck a small amount of an acid into the pipette and expel it again into the tube, repeat this twice. Mix the content quickly but gently with glass-rod for 10 min. Add Hcl drop by drop, mixing between each addition until the colour matches with standard. Read the amount of solution in the graduated tube, the calibrations give the Haemoglobin concentration.

$$\text{Calculation Hemoglobin concentration (gm/dl)} = \frac{\text{Abs. of Test}}{\text{Abs. of standard}} \times 16.31.$$

RBC count (Erythrocyte count)

Erythrocytes were counted by the method of Rusia and Sood (1992) [9] using haemocytometer.

Principle

The blood specimen is diluted with red cell diluting fluid which does not remove the white cells but allow red cells to be counted in a known volume of fluid. Finally, the number of cells in undiluted blood is calculated and reported as the number of red cells per cubic millimeter of whole blood.

Procedure

Blood was drawn in a clean RBC pipette up to its 0.5 mark. The tip of the pipette was wiped clean and dipped vertically into the red cell diluting fluid, which was then gently sucked up to mark 101. Then the tip of the pipette was closed with a finger and the contents were mixed thoroughly by shaking the pipette at right angles to its long axis. The red bead in the bulb helps for proper mixing of blood with the diluting fluid. The counting chamber of the haemocytometer was washed with distilled water, covered with a clean special cover glass and focused under a compound microscope. The ruled area of the haemocytometer was located clearly. Then the first drop of the fluid in the pipette was discarded by holding the pipette at 450 nm. The tip of the pipette was touched between the cover slip and the counting chamber and the diluted blood was applied by blowing. The blood was drawn into the chamber was left as such for 3 minutes to allow the cells to settle down.

Counting

The slide was first examined under low power and then under high power magnification. The counting chamber of the haemocytometer has a central heavy ruled area of 1 sq. mm. This central area is RBC counting chamber. It is divided into 25 squares and each square is sub-divided into 16 small squares. For the erythrocyte count, the cells falling with in and those touching the right and upper margin of the four corner squares and the central square (8.0 small squares) were counted. The total number of erythrocytes per cubic millimeter of whole blood was then calculated.

Calculation

$$\text{Erythrocytes} = \frac{\text{No. of erythrocyte X Dilution counted}}{\text{Area counted X Depth of fluid}} \text{ (million/cu.mm of blood).}$$

Dilution - 200

Area counted - 5 X 0.04 = 0.2 square mm

Depth of fluid - 0.1 mm

WBC count (Leucocytes counted)
Leucocytes were counted by the method of Rusia and sood (1992) [9] using haemocytometer.

Principle
Blood is diluted with acid solution which removes the red cells by haemolysis and also accentuates the nuclei of the white cells, thus the counting of the white cells become easy. Counting is done with a microscope under low power and knowing the volume of fluid examined and the dilution of the blood, the number of white cells per cubic millimeter in undiluted whole blood is calculated.

Procedure
Blood was drawn up to the 0.5 mark using a clean WBC pipette. Then the pipette was immediately kept in a watch glass containing WBC diluting fluid and it was drawn up to mark, taking care that no air bubbles included. The contents were mixed well by rotating the pipette between the palms of the hands. The white bead in the pipette helps for proper mixing of blood with the diluting fluid. The diluted blood was allowed to stand as such for 3 minutes for haemolysis of red cells to occur. Again the contents were mixed by rotating the pipette. After discarding the first few drops of diluted blood the counting chamber of the haemocytometer was charged with the fluid making sure that no air bubble were trapped between the cover slip and the chamber. The cells were allowed to settle down for a minute.

Counting
For the counting of leucocytes, the slide was examined under low power magnification of microscope. The neubaur's counting chamber is divided into two counting area which are ruled. Each counting chamber is divided into a total ruled area of 9 sq.mm. The area of each square is 1 sq.mm area of the 4 corner slide was used for the counting of leucocytes. The cells falling within the four corners square were counted and the total number of leucocytes per cubic millimeter of whole blood was calculated.

Calculation

$$\text{leucocytes} = \frac{\text{No. of leucocytes X Dilution method No. of}}{\text{Area counted X Depth of fluid}} \text{ (1000/cu.mm of blood)}$$

Dilution - 20
Area counted - 4 X 1 = 4 square.mm
Depth of fluid - 0.1 mm

Result
In the present study the haemoglobin content, RBCs and WBCs were studied in disease induced *Cyprinus carpio* using different concentrations of *Abutilon indicum* plant extract. Haemoglobin content on disease induced *Cyprinus carpio* fed with different concentration were (0.2ppm, 0.3ppm, 0.4ppm). In the normal control group fishes showed low level of haemoglobin content (6.52±0.1) when compared to control with Cadmium treated fish (5.40±0.15). The plant parts powder formulated diet treated fishes showed the gradually increased the haemoglobin content in different days of treatment. The highest content of haemoglobin was observed in 0.4ppm in (8.43±0.1) Cadmium mixture in plant diet treated fishes. The RBC count in the control fish was found to be 5.46±1.52 x 10^6 cells/ml on 8th day. The herbal treated fishes also showed more RBC count on 8th day *Abutilon indicum* 5.73±0.57 x 10^6 cells/ml. The RBC count in the control+ cadmium treated fish was found to be 6.10±1.0 x10^6 cells/ml. From this study, the maximum number of RBC cells were observed in 0.4ppm, when compared to other diet. The WBC counts were increased with increasing days of the treatment [0, 2, 4, 6 & 8 days], when compared to control + cadmium treated fish. The more number of WBC were present in control 4.53±1.52 on 8th day and also in plant treated fishes. Among the experimental groups, more WBC counts were observed in *Abutilon indicum* leaf treated fishes than the other experimental fishes.

Discussion
In this present investigation the signification decreased in the various parameter of blood was observed in Indian major carp *Catla catla* due to the treatment of different toxicant for short durations. The haematological factor in fish can much change in response to chemical stressors. However, there alterations are nonspecific to a range of substance. This study shows that mean haemoglobin in the control was 15.38. A decrease in the concentration of haemoglobin in blood is usually caused by the effect of toxic metals on gills, as well as decrease in oxygen, which also suggests anaemia or confirms toxic impact of copper and zinc in *Puntius parrah*. Haematological indices (RBC count, concentration of haemoglobin and haematocrit) have been reported to show secondary responses of an organism to irritants (Rogers *et al*. 2003) [10]. Changes observed in the haematological parameters can be used as an indicator of copper and zinc related stress in fish on exposed to higher level of these metals (Annune *et al*. 1994) [11], who also observed as a non-significant decrease in red cells for *O. niloticus*. The non-significant decrease in erythrocyte count and erythrocyte sedimentation rate of *Heteroclarias* sp. may be attributed to the swelling of red blood cells, such swelling of the red blood cells (erythrocytes) may be due to increase in protein and carbon dioxide content in the blood (Kori *et al*. 2008) [12]. Thus increasing or decreasing numbers of white blood cells were said to be a normal reaction, when zinc and cadmium demonstrating the effect of the immune system under toxic conditions (Flos *et al*. 1987) [13]. An increase in WBC was observed in the present investigation that was statistically significant. Earlier, Mishra and Stirastraya (1980) [14] also reported that leucocytes count were increased, when they exposed fishes to heavy metals. An increase in the metal concentration and exposure period resulted in the increased WBC, which was more pronounced in copper and zinc exposures.

Conclusion
We suggest that the herbal extract *Abutilon indicum* were effective in combating the toxic effect of heavy metals and more efficient in relieving the stress induced by heavy metals. Application of this herbal extract could be reduced by their incorporation into the leaf mixture to promote fish health. In this way aquatic species could be protected from the effect of heavy metals. Further exploration is needed to determine the response to *Abutilon indicum* in other fish varieties.

Table 1: Toxicity of cadmium and their effect on Haemoglobin content (g/dl) of common carp (*Cyprinus carpio*) exposed to crude leaf extract of *Abutilon indicum.*

Days	control fish	Name of the plant *Abutilon indicum* powder 20g/100g feed diet+ Cadmium chloride		
		0.2ppm	0.3ppm	0.4ppm
0 days	6.52±0.1	6.20±0.2	5.60±0.30	5.40±0.15
2days	6.90±0.5	6.00±0.05	6.52±0.1	7.20±0.2
4days	7.30±0.06	6.30±0.15	6.60±0.1	7.30±0.06
6days	8.34±0.1	6.50±0.20	6.90±0.52	7.93±0.15
8days	8.90±0.05	6.50±0.20	7.20±0.2	8.43±0.1

Table 2: Toxicity of cadmium and their effect on RBC count ($x10^6$ cells/ ml) of common carp (*Cyprinus carpio*) exposed to crude leaf extract of *Abutilon indicum.*

Days	control fish	Name of the plant *Abutilon indicum* powder 20g/100g feed diet+ Cadmium chloride		
		0.2ppm	0.3ppm	0.4ppm
0 days	5.46±1.52	5.50±1.00	5.60±1.0	5.73±0.57
2days	5.50±0.57	5.53±0.57	5.70±1.0	5.80±1.0
4days	5.66±0.57	5.66±0.57	5.80±1.0	5.90±0.57
6days	5.80±1.0	5.93±2.80	5.90±1.0	5.96±1.52
8days	5.96±0.57	5.63±1.15	6.0±1.0	6.10±1.0

Table 3: Toxicity of cadmium and their effect on WBC count ($\times10^3$ cells /ml) of common carp (*Cyprinus carpio*) exposed to crude leaf extract of *Abutilon indicum.*

Days	control fish	Name of the plant *Abutilon indicum* powder 20g/100g feed diet+ Cadmium chloride		
		0.2ppm	0.3ppm	0.4ppm
0 days	4.00±1.0	3.30±2.6	3.33±1.52	3.52±2.00
2days	4.10±1.0	3.26±1.52	3.40±1.0	3.50±1.15
4days	4.15±1.52	3.37±1.52	3.35±1.0	3.67±1.52
6days	4.37±1.15	3.47±1.52	3.60±1.0	3.60±1.0
8days	4.53±1.52	3.50±1.0	3.70±1.0	3.76±1.52

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