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## RESEARCH ARTICLE

# TOXICITY EFFECT OF THE DETERGENT TIDE ON THE HAEMATOLOGICAL PARAMETERS OF THE FRESHWATER FISH *CIRRHINUS MRIGALA*

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### ABSTRACT

The presence of detergent in water accelerates the corrosive action, impedes the filtering, sedimentation and coagulation processes, increases the saturation of water with oxygen and also deteriorates the taste properties of water. Soaps and detergents are one of the common pollutants, which causes pollution of inland water at tremendous pace and with the advent of potent anionic surfactant chemicals. The blood parameters were analysed in the fish, *Cirrhinus mrigala* and the LC50 value was determined when the fish was exposed to detergent tide. The parameters like RBC, Hb, MCV, PCV, Albumin and globulin were decreased and WBC have been increased in the fish.

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## INTRODUCTION

Blood is the most important and abundant body fluid. Its composition often reflects the total physiological activities. The main route of entry for any pesticide is through the gills. From the gills, it is transported to various parts of the body via the blood stream.

The haematological parameters have been considered as diagnostic indices of pathological conditions in animals. Fish blood can serve as a valuable tool in detecting physiological changes taking place in animal.

Masud *et al.* (2005) in *Cyprinus carpio* following mercuric intoxication. Kumar *et al.*, (2006) in *Clarias batrachus* and Singh and Singh (2007) in *Heteropneustes fossilis*.

The decrease in the RBC, Hb, PCV, MCV concentrations has been reported by Benarjee *et al* (2009) in the fish *Channa punctatus* to the rayon industrial effluents. Afaq *et al* (2010) reported a decrease in Haemoglobin concentration in freshwater teleost *Cirrhinus mrigala* when treated with leather dyes Bismarck brown and acid leather brown.

## MATERIALS AND METHOD

### Analytical Test for Water Chemistry

The tap water free from contaminants was used as dilution water for the present study. The physico-chemical analysis of water used in the experiments were carried out using the method of APHA (1998).

Physico-chemical parameters of the tap water used for the present study are as follows; Temperature  $27.2 \pm 0.9$  (°C), pH  $7.1 \pm 0.1$ , Dissolved O<sub>2</sub>  $5.9 \pm 0.4$  (mg/l), Alkalinity  $148 \pm 0.7$  (mg/l), Salinity  $0.4 \pm 0.1$  (ppt), Total Hardness  $190 \pm 1.9$  (mg/l), Calcium  $132 \pm 1.1$  (mg/l), Magnesium  $65 \pm 0.2$  (mg/l).

### Procurement and Maintenance

The fingerlings of the freshwater fish, *Cirrhinus mrigala* ranging in weight from 3g to 8g and measuring 4cm to 8cm in length) were procured from "Tamil Nadu Fisheries, Department corporation" Mettur, Salem District. The procured bulk samples of *Cirrhinus mrigala* were transported to the laboratory in well aerated polythene bag and acclimatized to the laboratory conditions under natural photoperiod for one week in large plastic containers at  $(26 \pm 5$  °C). The tank was

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previously washed with potassium permanganate to prevent any fungal infection. The fishes were maintained in dechlorinated tapwater of the quality used in the test and water was renewed everyday to provide freshwater, rich in oxygen.

Continuous artificial aeration was maintained throughout the acclimation and exposure periods. During this period the fish were fed with mixture of oilcake and rice bran. Unhealthy fish and those with infections were removed. Feeding was stopped two days prior to the experiment to maintain same state of metabolic requirements. Fish belonging to both sexes were selected for the present investigation. All the precautions, laid down on recommendations of the toxicity tests to aquatic organisms are followed (Anon 1975).

Technical grades of Tide an detergent was used in this investigation. Detergent Tide contains Alkyl Sulfate, Borax, citric acid, Diethylenetriamine pentaacetate (Sodium salt), Ethanolamine, FD & C Blue 1, Glycerin, Hydrogen peroxide, Liquitint™ Blue, Mannanase, Nonanoyloxybenzenesulfonate, polyethylene oxide, Sodium polyacrylate and Trimethoxy Benzoic Acid.

#### **Evaluation of Median Lethal Concentration (LC<sub>50</sub>)**

The concentration of the pollutant at which 50 percent of the test animals die during a specific test period or the concentration lethal to one half of the test population is referred to as median lethal concentration (Lc50) or median tolerance limit. In aquatic toxicology the traditional Lc50 test is often used to measure the potential risk of a chemicals (Jack de Bruijin *et al.*, 1991).

Batches of 10 healthy fishes were exposed to different concentrations of detergent Tide to calculate the LC50 value. One more set of fishes are maintained as control in tap water. To find the wide range of concentration 10-100 mg were chosen and the number of dead or affected fishes in each setup was counted at regular intervals upto 48 hrs. The level of the dissolved oxygen, pH, alkalinity and hardness were monitored and maintained constant.

Appropriate narrow range of concentration 10-50 mg was used to find the median lethal concentration, using a minimum of 6 fishes, for each concentration and the mortality was recorded for every 24 hrs upto 72 hrs. It was found as 36mg for 48 hrs, using probit analysis method (Finney, 1971). From the stock solution various sub-lethal concentrations were prepared for bioassay study.

Three groups of fishes were exposed to 3.6 mg (1/10<sup>th</sup> of 48 hrs LC50 value) concentration of the detergent 'Tide' for 24, 48 and 72 hrs respectively; Another group was maintained as control. All the groups received the same type of food and other conditions were maintained similarly. At the end of each exposure period, fishes were sacrificed and tissues such as liver, gill, muscle, brain and kidney were dissected and removed. The tissues (10mg) were homogenized in 80% methanol, centrifuged at 3500 rpm for 15 minutes and the clear supernatant was used for the analysis of different parameters.

#### **Estimation of Haemoglobin Content (Hb)**

The haemoglobin content was estimated by acid haematin method (Sahli, 1962).

#### **Counting of Red Blood Corpuscles (RBC)**

Total Red blood cells (RBC) were counted using the improved Neubaur haemocytometer (Shah and Altindag, 2004). Blood was diluted (1:200) with Hayem's Fluid (Mishra and Pandey, 1977). Erythrocytes were counted in the loaded haemocytometer chamber and total numbers were reported as 10<sup>6</sup> x mm<sup>3</sup> (Wintrobe, 1967).

#### **Counting of White Blood Corpuscles (WBC)**

Total White blood cells (WBC) were counted using an improved Neubaur haemocytometer (Shah and Altindag, 2005; Mgbenka and Oluah, 2003).

#### **Calculation of mean corpuscular volume (MCV)**

The mean corpuscular volume was calculated by using values of PCV % and the red blood cell counts and expressed in  $\mu\text{m}^{-3}$  (Anderson and Klontz, 1965).

#### **Determination of Packed Cell Volume (PCV)**

Blood was sucked into the heparinized haematocrit capillary tube (7.5cm length, 0.1cm width). After sealing both the sides of the tube it was centrifuged in the microhaematocrit centrifuge at 6000 rpm for 2 min. From the volume of blood taken and cell volume after centrifugation, the PCV percentage was calculated employing standard method and formulae (Sandhu, 1990).

## **RESULTS AND DISCUSSION**

The amount of RBC, WBC, Hb, MCV, PCV, Albumin, Globulin estimated in blood of the fish *Cirrhinus mrigala* subjected to different exposures, are presented in the form of tables (1)

The amount of RBC was found to contain 2.12, 2.00, 1.89, 1.75 x 10<sup>6</sup>/cu.mm, in WBC it was recorded as 18.51, 17.41, 16.52, 14.12 x 10<sup>3</sup>/cu.mm, the level of Haemoglobin was noted as 5.80, 4.90, 4.10, 3.00 gms/dl, the values of MCV as 75.20, 84.40 64.60, 57.80  $\mu\text{m}^3$ , the results of PCV as 6.30, 6.00, 5.30, 4.70%, the level of Albumin was recorded as 2.05, 1.35, 1.15, 1.02 mg/dl, the level of Globulin was analysed as 1.09, 0.99, 0.97 and 0.95 mg/dl in control, 24 hrs, 48 hrs and 72 hrs respectively.

The values were decreased from control in RBC, WBC, Hb, MCV, PCV, Albumin and Globulin. The above results were statistically analysed by ANOVA one way and most of the values were found to be significant at 5% level. Blood often exhibit pathological changes before the appearance of any external symptom of toxicity.

**Table 1** Effect of detergent Tide on different parameters in blood of the fish, *Cirrhinus mrigala*.

S.No	PARAMETERS	CONTROL	24 HRS	48 HRS	72 HRS	CD (5%)
1.	Total erythrocyte count (TEC) 10 <sup>6</sup> /cu.mm	2.12 <sup>a</sup> ± 0.08	2.00 <sup>b</sup> ± 0.41	1.89 <sup>a</sup> ± 0.24	1.75 <sup>a</sup> ± 0.16	0.58
2.	Total leucocyte count (TLC) 10 <sup>3</sup> /cu.mm	18.51 <sup>a</sup> ± 0.08	17.41 <sup>b</sup> ± 0.16	16.52 <sup>c</sup> ± 0.10	14.12 <sup>d</sup> ± 0.04	0.243
3.	Haemoglobin (Hb) (gms/dl)	5.80 <sup>a</sup> ± 0.33	4.90 <sup>b</sup> ± 0.16	4.10 <sup>c</sup> ± 0.24	3.00 <sup>d</sup> ± 0.41	0.692
4.	Mean corpuscular volume (MCV) (µm <sup>3</sup> )	75.20 <sup>a</sup> ± 0.82	84.40 <sup>b</sup> ± 1.63	64.60 <sup>c</sup> ± 0.49	57.80 <sup>d</sup> ± 0.65	2.306
5.	Packed cell volume (PCV) (%)	6.30 <sup>a</sup> ± 0.16	6.00 <sup>b</sup> ± 0.08	5.30 <sup>c</sup> ± 0.04	4.70 <sup>d</sup> ± 0.12	0.257
6.	Albumin (mg/dl)	2.05 <sup>a</sup> ± 0.08	1.35 <sup>b</sup> ± 0.04	1.15 <sup>bc</sup> ± 0.12	1.02 ± 0.16 <sup>c</sup>	0.253
7.	Globulin (mg/dl)	1.09 <sup>a</sup> ± 0.16	0.99 <sup>a</sup> ± 0.07	0.97 <sup>a</sup> ± 0.08	0.95 <sup>a</sup> ± 0.04	0.228

Values are mean ± SD of three observations

% : Percentage increase/decrease over control

In a column, means followed by a common letter are not significant at the 5% level by DMRT

Therefore, the haematological studies in animal form a promising tool for the investigation of physiological alteration caused by environmental pollution. The results of the present study showed significant decrease in RBC, WBC, Hb, MCV, PCV, Albumin and Globulin in the tissues studied. The percent decrease was found to be more in Albumin (50.24%) and less in PCV (4.76%) (Table 1).

Similar reduction of blood parameters were studied by various authors. Pooja Gupta and Kumar saxena, (2006) observed increased WBC counts in *Channa punctatus* after exposure to cyhalothrin and permethrin. Anil Kumar and Janaiah (2009) have reported decrease in albumin in the fish *Channa punctatus* when exposed to imidacloprid. Jayaseelan *et al.* (2011) have noted that the RBC count decreased significantly in the fresh water fish, *Labeo rohita* on exposure to herbicide glyphosate. Soundararajan and Veeraiyan, (2014) have noticed the decreased haemoglobin content in the fresh water fish, *Tilapia mossambica* on exposure to arsenic. Sachar and Raina, (2014) have observed the decreased haematocrit value (PCV) in the fish, *Aspidoparia morar* on exposure to lindane. All these observations confirm the findings of the present study.

In fish, the reduction in leukocyte number may be due to the stress associated leucopenia (Pickford *et al.*, 1971; Johanson and Larsson, 1978) as a result of an increase in corticosteroids. The decrease in RBC and Hb concentration indicates acute anaemia. The anaemia could be due to the destruction of RBC. The anaemia may also be of haemolytic type.

In the present investigation, haemolysis might have been one of the causes for reduction in Hb, RBC and PCV values. The fall in haematological parameters might be due to decreased rate of production and or to an increased loss of destruction of RBC (Larson, 1975). The another reason for RBC suppression could also be damage to the haemopoietic tissue, PCV appears to be positively correlated with RBC counts, hence, a decrease in PCV is observed.

Increase in MCV values also indicates endosmosis which involves passage of solvent from a less concentrated solution to more concentrated solution (Haemodilution). The decrease in albumin indicates fall in osmotic pressure leading to enhanced fluid retention tissue spaces causing edema in animals. Reduction in haemoglobin concentration may also be due to hypohaemoglobinemia.

## CONCLUSION

RBC showed maximum decrease of 17.45 % in blood after 72 hrs exposure and minimum of 5.66 % after 24 hrs exposure in 36 mg **Tide**.

WBC showed maximum decrease of 23.71 % in blood after 72 hrs exposure and minimum of 5.94 % after 24 hrs exposure in 36 mg **Tide**. Haemoglobin showed maximum decrease of 48.27 % in blood after 72 hrs exposure and minimum of 15.51 % after 24 hrs exposure in 36 mg **Tide**.

The highest percent decrease 23.13 % in blood after 72 hrs exposure and minimum 12.23 % after 24 hrs exposure were noticed in MCV. The highest percent decrease 25.39 % in blood after 72 hrs exposure and minimum 4.76 % after 24 hrs exposure were noticed in PCV. The highest percent decrease 50.24 % in blood after 72 hrs exposure and minimum 34.14 % after 24 hrs exposure were noticed in Albumin. The highest percent decrease 12.84 % in blood after 72 hrs exposure and minimum 9.17 % after 24 hrs exposure were noticed in Globulin.

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