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Hematological, Biochemical and Immunological Responses of Estuarine Catfish *Mystus gulio* (Hamilton 1822) After Exposure to Lead (Pb)

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Abstract: Due to increased industrialization in developing countries some of the toxic substances are being increased in day-to-day life. Living organisms need some trace amounts of metals like copper, manganese, zinc etc. but some of the non-essential toxic heavy metals are also accumulated in the body of living organisms which may cause damages to the immune organs of the organism. The present study investigated the impacts of sublethal dosage of inorganic Lead acetate on the hematological, biochemical and immunological responses of the brackish water fish *Mystus gulio*. The LC_{50} at 96 h was determined by the Probit method. The experiment was performed for 30 days of exposure of lead acetate (4 mg/l) to the fish *Mystus gulio*. After exposure to lead nitrate hematological (hemoglobin, RBC, WBC, PCV, neutrophil, eosinophil and platelet), biochemical (glucose, protein, albumin and globulin) and immunological (IgM) parameters were analyzed. A decrease was seen in the RBC count, hemoglobin, PCV, MCV, MCH, MCHC, protein, albumin and globulin whereas an increase was seen in WBC count, neutrophils, eosinophils, ESR, platelet count, glucose and Immunoglobulin (IgM).

Keywords: *Mystus gulio,* Lead, RBC, WBC, Hematology, Biochemical, Immunology, Immunoglobulin

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Introduction

In recent years the wastewater discharged into the aquatic water systems without proper treatment of unwanted and toxic substances including heavy metals affects the healthy life of aquatic organisms as well as humans. Some of the heavy metals (HM) Iron, Zinc, Manganese, etc. are useful at the same time some of the HM like Lead, Cadmium, Mercury, etc. are non-essential and toxic and produce adverse effects on the organisms. The anthropogenic resources produce heavy metals (Bauvais *et al*., 2000). Water is the biological solvent and integral component of living things. Million tons of heavy metals, pesticides and fertilizers are used for industrial and agricultural purposes. These are entering into the water bodies through surface runoff and drain into the rivers and streams. Industrial waste and inorganic wastes pose threats to fishes and other organisms (Patil and Dhanda, 2000; Shin, 2002). The heavy metal deposit in the aquatic sources through geological weathering, direct atmospheric deposit, municipal, agriculture and the evacuation of residential wastes (Maier *et al*., 2014; Dhanakumar *et al*., 2015; Garcia *et al*., 2015). Heavy metal accumulation in the tissue depends on the concentration of HM in water and exposure duration. Albeit some other environmental factors, for example, in the fish O_2 concentration, pH, salinity, hardness, alkalinity, dissolved carbon dioxide and water temperature may influence and play a vital role in heavy metal toxicity (Jitar *et al*., 2014). In the aquatic food chain, fish is the last existing one and heavy metals accumulate and pass through the food chain to human beings affecting them acute and chronically (Al-Yousuf *et al*., 2000). Pollutant accumulates in tissue which depends on the level of the metals in the water and the exposure period. HM accumulate in the fish directly from the aquatic environment or aquatic organisms like zooplankton, crustaceans and small fish indirectly (Polat *et al*., 2015). The HM enter into the bloodstream then traverse to the tissues and organs and it precipitates in all organs (Fazio *et al*., 2014; Abdel-Wahab *et al*., 2020).

Lead is used in petroleum refineries, paints, batteries, insulation sheets, pipes, electronic products etc. In long- and short-term effects, Pb accumulation cause severe effects and diseases in human, and other organisms resulting into memory loss, learning disorders, and other threatening diseases. The ingestion and inhalation are the root cause of the absorption of the Pb in various organisms (Verma *et al*., 2020). Pb is persistent heavy metal, described as the most hazardous substance (Sfakianakis *et al*., 2015). Lead bioaccumulation in fish tissues and blood cause damages which may lead to disorders in the organs of the fish (Srivastav *et al.*, 2013; Rai *et al*., 2013). The specific symptom of Pb toxicity includes acute damage and changes in RBC, WBC, blood variables which affect the nervous system (El-Badawi 2005; Ikeogu *et al*., 2016: Khalesi *et al*., 2017). Even at minimum concentration the Pb, Cd and other HM are toxic and highly injurious in long time exposure (Guardiola *et al*., 2013).

The dosage and time duration cause changes in haematology, neurology, reproductive, gastrointestinal, circulatory, histology and immune response (Nimmy and Pawlin 2018). Haematological parameters act as an indicator of physiological changes in the fish (Satheeshkumar *et al*., 2011).

Mystus gulio is a freshwater fish, delicious and of high consumer value with market demand in Asian countries, especially in India. *Mystus gulio* can be used as a biomarker organism. However, few studies demonstrate the change in haematological, biochemical and immune responses in catfish exposed to lead. Hence, the present study aimed to investigate the haematological, biochemical and immunological responses in catfish *Mystus gulio* after exposure to the sublethal concentration of lead nitrate.

Materials and Methods

Collection of fish:

Healthy fish *Mystus gulio* (18±6 g; 10±4 cm) were collected from Vellar estuary, Parangipettai, the Southeast coast of Tamil Nadu, India. After collection the fish were cleansed with 0.02% KMnO4 solution. Fish were acclimatized for 15 days and during period they were fed with pieces of tissues and soy meal. The selected fish were kept in the fibre tank (90×40×50 cm) with an adequate supply of aeration in chlorine-free water. The water quality parameters were maintained with photoperiod in the experimental duration. All debris was removed regularly.

Experimental design:

Lead nitrate ($Pb(NO_3)_2$) was purchased from Hi media, Mumbai and used in static bioassay. 96h LC_{50} value was found as 400 ppm for lead nitrate. The LC_{50} value was calculated using the Probit analysis (Finney, 1964) method. After determination of LC_{50}, ten fish were placed in the fibre tank containing 20 L of water. One tank served as control, the other one served as experimental. The triplicate method was followed for the experiments. The set of ten fish in the experimental tank was exposed to sublethal concentration of 4 mg/l lead nitrate (i.e. 1/10th of 96 h LC_{50}). The concentration of the toxic substances was renewed every day. After 30 days of duration period, the hematological, biochemical and immunological parameters were determined.

Estimation of Hematological parameters:

After 30 days of exposure fish were anaesthetized using clove oil and the blood was collected from the caudal vessel of the fish using heparinized disposable syringes and poured into the Eppendorf tubes coated with EDTA and stored for the biochemical, hematological and immunological analyses. Hemoglobin, RBC, WBC, PCV, Neutrophil, Eosinophils and Platelets counts were performed with whole blood of control as well as treated fish. Erythrocyte variables MCV, MCH, MCHC and ESR of the fish were also determined. To estimate hematological parameters basic standard procedure (Blaxhall and Daisley, 1973) was followed. For RBC and WBC count, an improved Neubauer Haemocytometer counting chamber was used. 3 g of sodium nitrate with 1 ml of formalin and 99 ml distilled water was used to dilute the blood, Blood parameters were counted at the centre and corners of the slide under the light microscope. Hemoglobinometer was used to eastimate the Hb content. All erythrocyte variables were estimated using method of Dacie and Lewis (1977). Micro-haematocrit was used to determine haematocrit (Dacie and Lewis, 1977; Baker and Silverton, 1982). To calculate leukocytes differential count using Wright-Giemsa stain was used, and for counting platelets the protocol suggested by Snieszko (1960) and Ghaffar *et al*. (2017) was followed.

Estimation of Biochemical Parameters:

Estimations of glucose, protein, albumin and globulin in the whole blood of control and experimental *M. gulio* were performed. Blood sample was taken and left to coagulate at room temperature for 15 min, after coagulation the blood was centrifuged at 3000 rpm for 20 min to get the serum. The biochemical parameters were analyzed by standard method (Nimmy and Pawlin, 2018) as follow:

(i) The estimation of glucose was calculated by phosphomolybdate method as-

mg of glucose/100ml of blood = mg of glucose in standard X (OD value of test/ OD value of standard) ×100/0.2

(ii) The estimation of protein was performed by Lowry *et al.* (1951) method.

(iii) The estimation of albumin by Bromocresol green method as:

Albumin (g/dl) = A test/ A standard × concentration of standard

Where A= absorbance

(iv) The estimation of globulin by Bromocresol green method as:

Serum globulin level (g/dl) = Serum total protein – serum albumin

(v) The estimation of Immunoglobulin (IgM) by standard ELISA assay procedure (Nimmy and Pawlin, 2018).

Statistical analysis

The significance between control and experimental fish were determined by Student's t-test. The mean ± standard error has been analyzed with IBM SPSS 16.0 software version and the differences were considered significant at $P<0.05$.

Results

Hematological Parameters:

The effects of lead nitrate on hematological and biochemical parameters of the fish *M. gulio* has been illustrated in Tables 1, 2 and 3. Hemoglobin (Hb) content of the control fish was 10.15±0.23 g/dl whereas in lead treated fish a significant ($P<0.05$) decrease (9.82±0.53 g/dl) was recorded (Table 1; Fig. 1). A decrease was noticed in RBC count in experimental fish ($5.47\pm0.12 \times10^6$ /mm^3) as compared to control ($5.92\pm0.06 \times10^6$ /mm^3) (Table 1; Fig. 1).

After 30 days lead exposure the WBC count has been increased (control $29.43\pm0.86 \times10^3$/

Table 1: Hematological parameters after 30 days in control and lead treated fish *M. gulio*

Haematological Parameters	Control	Treated
Hb (g/dl)	10.15±0.23	9.82±0.53
RBC ($\times 10^6$ /mm^3)	5.92±0.06	5.47±0.12
WBC ($\times 10^3$/mm^3)	29.43±0.86	31.65±0.82
PLT ($\times 10^3$/mm^3)	37.38±1.12	35.43±1.04
PCV (%)	23.92±1.13	21.43±1.07
Eosinophil (%)	50.47±8.72	52.60±0.67
Neutrophil (%)	2.18±1.02	2.28±0.19

Haemoglobin - Hb, Reb Blood Corpuscles - RBC, White Blood Corpuscles - WBC, Platelets - PLT, Packed Cell Volume - PCV

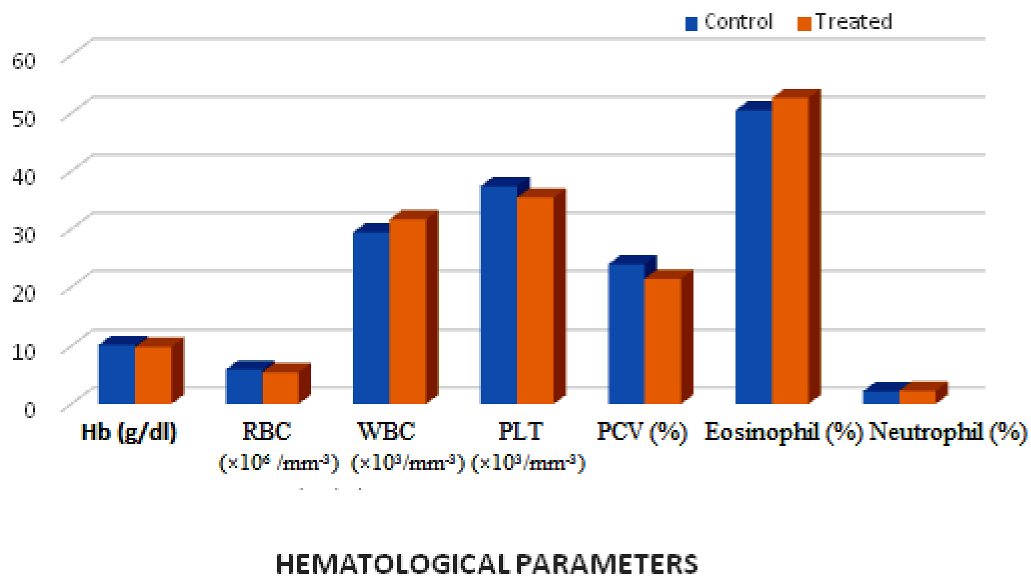

Fig 1: Hematological parameters after 30 days in control and lead treated fish *M. gulio.*

mm^3; lead exposed 31.65±0.82 $\times 10^3$ /mm^3)(Table 1; Fig. 1). The platelet count was noticed to be decreased (control 37.38±1.12 $\times 10^3$ /mm^3; lead exposed 35.43±1.04 $\times 10^3$ /mm^3) in 30 days lead treated fish (Table 1; Fig. 1).

Packed cell volume (PCV) after 30 days lead treatment showed a decrease (Table 1; Fig. 1). The eosinophils and neutrophils count of lead exposed fish showed an increase as compared to control (Table 1; Fig. 1).

Erythrocyte Counts:

The MCV and ESR values of 30 days lead treated fish exhibited an increase as compared to control (Table 2; Fig. 2) whereas MCH and values decreased in lead treated fish (Table 2; Fig. 2)

Biochemical Parameters

The albumin (ALB), globulin (GLB) and total serum protein (TP) contents of control fish have been recorded as 2.23±0.06 (g/dl), 1.18 ± 0.05 (g/dl) and 4.76±0.17 (g/dl), respectively whereas in 30 days lead exposed fish the values have been noticed to be decreased as 1.45±0.05 (g/dl), 0.48±0.02 (g/dl) and 3.04±0.22 (g/dl), respectively (Table 3; Fig. 3). The values of glucose and IgM of lead treated fish exhibited an increase as compared to control fish (Table 3; Fig. 3).

Table 2: Erythrocyte counts of control and lead treated fish *M. gulio* after 30 days

Erythrocyte Counts	**Control**	**Treated**
MCV (fl)	62.21±1.32	63.56±1.42
MCH (pg)	17.41±0.08	16.01±0.02
MCHC (g/dl)	36.12±0.05	35.87±0.18
ESR (mm/h)	26.19±1.02	28.03±0.56

Mean Corpuscular Volume - MCV, Mean Corpuscular Haemoglobin - MCH, Mean Corpuscular Haemoglobin Concentration - MCHC, Erythrocyte Sedimentation Rate - ESR.

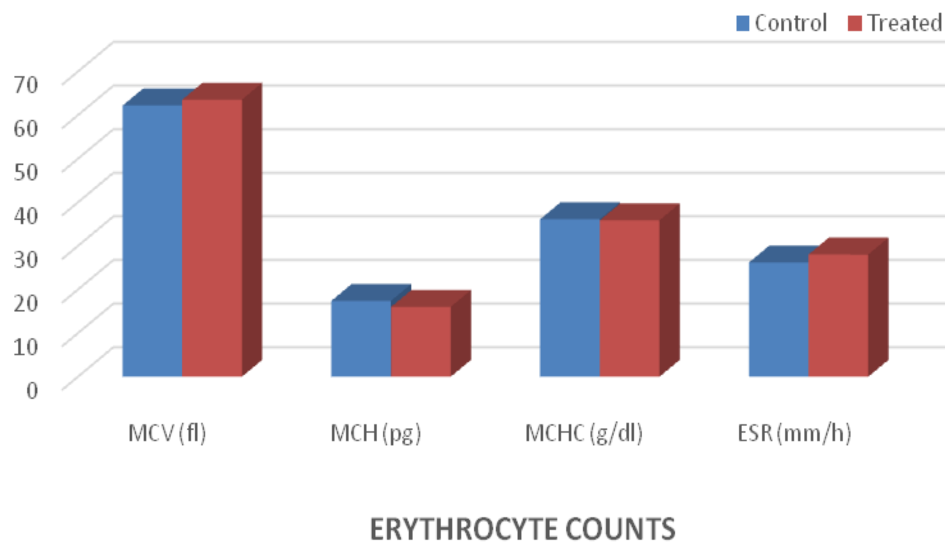

Fig. 2: Erythrocyte counts of control and lead treated fish *M. gulio* after 30 days.

Table 3: Biochemical parameters of control and lead treated fish *M. gulio* after 30 days

Biochemical Parameters	**Control**	**Treated**
Albumin (g/dl)	2.23±0.06	1.45±0.05
Globulin (g/dl)	1.18 ± 0.05	0.48±0.02
Total glucose (mg/dl)	57.39±3.38	60.21±4.08
Total protein (g/dl)	4.76±0.17	3.04±0.22
Immunoglobulin (μg/ml)	7.28±0.08	10.15±0.32

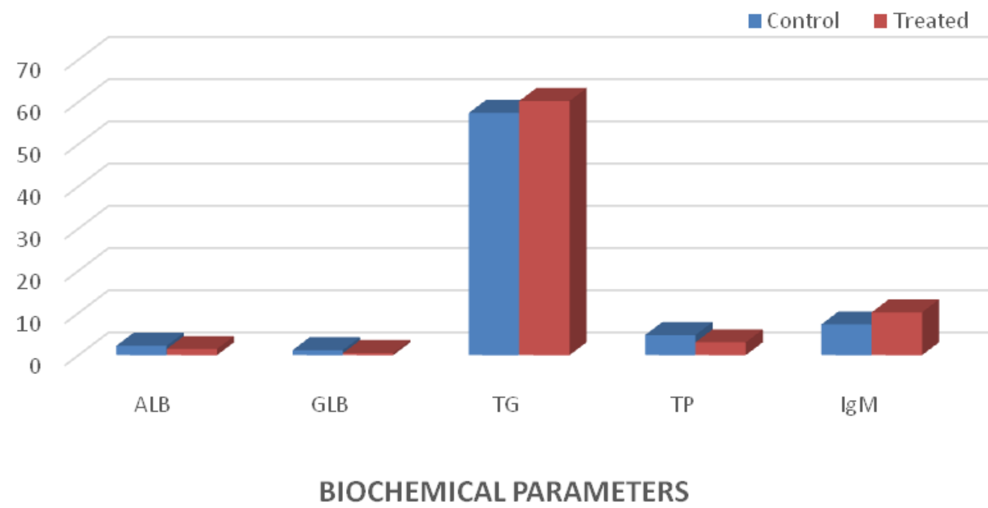

Fig. 3: Biochemical Parameters of control and lead treated fish *M. gulio* after 30 days.

Discussion

Aquatic organisms are directly in contact with contaminated water. In the fish, toxic metal concentration in tissue is a good indicator of water contamination by metal exposure (Carla *et al*., 2004; Al-Kahtani, 2009). The major role of RBC is to transport hemoglobin which plays a vital role to transport oxygen to the body tissues (Hibiya, 1982). The RBC counts diminished after exposure to lead could be the consequence of annihilation or hemolysis of red corpuscles. The hemopoietic organs are not able to form normal red blood cells and, in this way there was decrease in RBC due to metal toxicity. Internal bleeding in the damaged kidney may be the cause for decrease in the RBC count. In *Heteroclarius* similar findings were recorded in the past after sublethal concentration of Cd exposure (Kori-Siakpere *et al*., 2006). This supports the findings of the present study. The decreased Hb content may be due to the concealment of the haemopoietic action of the kidney and to expand the expulsion of dysfunctional red blood cells following metal exposure.The decrease in Hb in fish subjected to toxic exposure could be because of the impact of inhibitory action of toxicant on the enzyme activity responsible for the Hb synthesis (Mustafa, 2012). The present study supports the findings that the decrease of erythrocytes and Hb count leads to hypoxic and hemopoiesis destruction due to exposure to heavy metals.

The antibody production has been increased which helps to a significant increase of white blood cells count thus cause survival recovery of the fish in response to metal exposure (Joshi and Deep, 2002). The WBC is responsible for different stress activities which includes infection and synthetic aggravations in the fish (Christensen *et al*., 1978). The stimulation of lymphopoiesis increase the production of lymphocytes from lymphoid tissues. The lymphocytes response to toxic pollutants is associated with induced extreme tissue defects and disturbance of non-specific immune response which leads to excess production of leucocytes (Das and Mukerjee, 2003). The findings of the present study strongly support these statements that an increase in WBC count and lymphopoiesis stimulation is due to heavy metals exposure. Fish blood contains WBC variants such as neutrophils, eosinophils, neutrophils, lymphocytes, and monocytes. In fish these cells may increase significantly due to HM exposure and these results reflect the prominent immunity in exposed *M. gulio*. The reports of other investigators in *Oreochromis niloticus* provide strength to the findings of the present study (Lawrence, 1981; Khan *et al*., 2020).

PCV correlate with erythrocyte concentration. A decrease in RBC may cause anaemia. In the present study there was decrease in erythrocytes and Hb which is due to lead toxicant. The reduction of RBC, PCV and Hb may cause hemolytic anaemia in fish exposed to sublethal concentrations of Pb. During stress conditions the fish release epinephrine which may destruct the deformed or premature production of RBC and may possibly disturb the haematocrit percentage. The present study is in conformity with the findings of Nazish Shah *et al*. (2020) as they have suggested that the decline of MCV may result in immature production of RBC from hematopoietic tissue when fish were exposed to Cd metal. The release of immature cells is to compensate for the reduction of RBC (Houston and Keen, 1984). Hypochromic microcytic anaemia is the symptom of declined MCV (Shakoori *et al*., 1996). Our findings derive support from the observations of Shakoori *et al*. (1996).

The MCH was decreased significantly when fish was exposed to toxicant (Atamanalp *et al*., 2002). The values were reduced in treated fish in the present study. To diagnose the amount of RBC, MCHC is the source of measurement (Milligan and Wood, 1982). MCV and MCH decrease may be directly related to hematocrit, RBC and Hb observed in treated fish. After exposure to metals, leucocyte counts may associate to the decline of non-specific immune response in the treated fish (Brandao *et al*., 2009). HM affects lesions and inflammations in tissue which cause anaemia, neoplasia and tissue damage. The macrocytic anaemia is caused by an increase in ESR (Sinha *et al*., 2000). These findings supports the observations of the present study (Adeyemo *et al*., 2008; Singh *et al*., 2008; Pal and Trivedi, 2016).

The most important protein content is albumin which plays a vital role in the osmosis of the blood content. Under toxic situations, the fish may adapt to the changes in serum albumin (Ibrahim, 1992). The decrease in albumin content in *Tilapia nilotica* after treatment could be responsible as an adaptive immunity and to detoxify the toxic substance (Sogorb *et al*., 2002). In stress and diseased conditions, serum albumin is the antecedent for the production of IgM and plays a significant role in the immune response of the living organisms. Subsequently, the IgM is called defensive proteins. Under the toxic conditions, the serum globulins were exceptionally high in the fishes. After exposure of atrazine the serum globulin level was critically decreased in *Oreochromis niloticus* and *Carassius auratus* (Banaee *et al*., 2011). The present study is in conformity with findings of other investigators who have reported decrease of serum globulin in the fish treated with toxicants (Banaee *et al*., 2011; Yekeen and Fawole, 2011).

In the treated fish *M. gulio* there is an elevation in serum glucose level when compared to control. Under stressful condition in fish the production of catecholamine and glucocorticoids increased rapidly from adrenal tissue which provoke hyperglycemia (Singh and Srivastava, 1982). In muscle and liver, hyperglycemia may be the product of glycogenolysis (Schreck, 1981). Reduction in serum protein might be due to decreased protein synthesis and disabled function of kidney (Ravichandran *et al*., 1994; Kumari and Kumar, 1995). The decrease of protein might be because of elevated proteolytic movement and the diminished anabolic action of protein (Jenkins *et al*., 2003; Radha *et al*., 2005).

In the present study IgM values were increased in response to Pd intoxication to fish. IgM values were reported to be higher in fish after exposure to lead and cadmium (Zaki *et al*., 2010). In the innate and adaptive immune response, the IgM is the contributor to immunity in fish. IgM plays a vital role in phagocytic activity to remove pathogens at cellular level (Ye *et al*., 2013).

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