Lead Acetate Induced Haematological Alterations In Indian Common Carps, Labeo Rohita (Rohu) And Catla Catla (Catla), Remediating With Heavy Metal Detoxification Diet

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Abstract
The aim of the study was to evaluate the effects of Lead acetate on Haematological alterations Indian common carps *Labeo rohita* and *Catla catla*, exposed to heavy metals i.e. Lead acetate. The results of the study concluded that the Heavy Metals Detoxification Diet (HMDD)fed to *Catla catla* showed significantly improved the haematological performance of fishes, exposed with different concentrations of Lead acetate . The potential of improving Haematological parameters in fish may be due to the nutritional and its antioxidant property. So, the present study recommends the use of HMDD in fish feed in appropriate amount as it reduces the toxicity of heavy metals in fishes.

Keywords: *Labeo rohita* and *Catla catla*, Heavy Metal Detoxification Diet, Haematology.

INTRODUCTION:

India is one of the main nations that produce textiles and garments clothing. Ecological contamination and the risks presented by the material industry are turning into a main issue. Material effluents contain an assortment of manufactured colours along with poisonous synthetic compounds, for example, acids, sulphur, salts, naphthol, nitrates, hydrogen peroxide, surfactant-scattering specialists, and toxic heavy metals (THM) like Cu, Cr, Cd, Zn, Ni, As, and Pb, which are delivered straightforwardly into water streams ***(Senthilkumar and Saravanan, 2017)*** and present danger to the oceanic living beings .***(Almroth et al., 2021).*** Lead (Pb) are poisonous metals that keep on drawing much consideration since they are inclined to be collected in fish tissues and can hurt humans whenever taken up with food. Long-haul openness to Pb causes poisoning in people and different vertebrates.Lead is unsafe to various organs and tissues, including the heart, bones, digestion tracts, kidneys, conceptive, and neurological frameworks. Among all, Cr and Pb are THM supported as major ecological pollutants aecotoxic elements ***(Duffus, 2002***). Evidence suggests that fish exposed to Cr and Pb lead to various toxicities ***(Shah et al., 2020)***. Being one of the regularly used metals, Cr and its particulates enter the aquatic medium through effluents released from various industries like textiles, tanneries, electroplating studios, mineral mining, dyeing, printing-visual, and medical industries. Evidence suggests that fish exposed to Cr and Pb lead to various toxicities ***(Shah et al., 2020)***. Medicinal plants encompass various types of plants utilized in herbal medicine. These plants also serve as food, sources of flavonoids, medicine, or perfumes and play a role in certain spiritual activities. As defined by the World Health Organization, a medicinal plant is any plant that contains substances in one or more of its organs/parts that can be used for therapeutic purposes. The whole plant of Cassia tora is of medicinal importance. The Cassia tora seed extract shows the presence of various phytoconstituents such as flavonoids, alkaloids, triterpenoids, and steroids. The extract showed maximum antioxidant activity. Antioxidants present in fruits, vegetables, and herbal plants are the main factor that reduces toxicity in cells.

MATERIALS AND METHODS

Preparation of Methanolic extract of *Cassia tora:*

The *Cassia tora* seeds were thoroughly washed with tap water followed by distilled water to remove dirt and dust. Freshly collected seeds (250 gms) of *Cassia tora* were powdered and dissolved in 3 litres of methanol in a separating funnel for 72 hours. The mixture was then subjected to maceration, and the soaked seeds were macerated and defatted using 2 litres of petroleum ether solvent. The extract was then recovered in an incubator at a low temperature of 40°C for 2 days, and the crude extract was scraped. Finally, 310 gms of dried crude extract was obtained and stored for various tests and herbal diet preparation. *Cassia tora* extract were mixed into the basal diet and formed into pellets.

Collection of fishes Catla

The Rohu and Catla fish, with an average size of 10-15 cm and weight of 28-30 gms, were obtained from a local shop in Jahangirabad, Bhopal. They originated from a hatchery in Calcutta. The fishes were housed in standard tanks and maintained in an air-conditioned animal house at Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal, with a temperature range of 30°C to 37°C and a relative humidity of 70-75%. The animal house operated on a 12-hour light-dark

cycle and followed the rules and regulations of the laboratory at Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal. The fishes were kept in well-aerated aquaria filled with 15L of water. The whole experiment was conducted under controlled laboratory conditions with three replicates each for 28 days. The fishes were fed with market feed and were starved 24 hours prior to the experiment. Physio-chemical parameters of water, including pH, temperature (°C), dissolved oxygen (mg/litre), total hardness (mg/l), and alkalinity (mg/l), were monitored and recorded daily throughout the experiment. Water temperature was measured using a thermometer, pH was measured using a pH meter from, and DO was measured using a DO meter.

Grouping of fishes Rohu and Catla

Catla fishes, aged 2 to 3 months, with an average size of 10-15 cm and weight of 28-30 gms, were divided into 4 groups, with each group containing 8 fishes (4 Rohu, 4 Catla).Group -1 was further divided into subgroups A, B, C, receiving various concentrations of Lead Acetate, serving as the Positive Control Group. Group 2 received Lead Acetate with various concentrations and were fed with HMDD (Heavy metal detoxification diet), serving as the Test Group. Group 3 received only HMDD (Heavy metal detoxification diet), serving as the Test Control. Group 4 received only regular diet, serving as the Standard Control.

Lead: Lead Acetate
Dose 1. 5mg/litre for 15 to 28 days (A)
Dose 2. 15mg/litre for 15 to 28 days (B)
Dose 3. 30mg/litre for 15 to 28 days(C)

Hematological in blood samples of Rohu and Catla fishes by Jan etal., method.

Statistical Analysis: The values shown are Mean ± SEM
Significance: $p < 0.05$, compared to control (one way ANOVA followed by Tukey Kramer/ Tukey HSD multiple comparison tests).

TC vs A-1, A -2, B -1, B -2, C -1, C -2
A-1vs A -2, B -1 vs B2, C-1 vs C-2
STC vs A-1, A -2, B -1, B -2, C -1, C -2
Variation among rows means is significantly greater than expected by chance.
Tukey -Kramer multiple comparison test.

RESULTS AND DISCUSSION

Haematological alterations have been shown cellular and nuclear abnormalities in Rohu and Catla fishes exposed to different concentrations of heavy metal. The toxicity of metal and the effect of HMDD (Heavy metal detoxification diet) studied on the 15th and 28th days of the experiment on Rohu and Catla fishes the blood samples (1.5–2 ml) from the vein of fishes. After collection of blood, the blood was immediately transferred to EDTA anticoagulant for measurement of Red blood cells (RBCs)White blood cells(WBCs), Haemoglobin, observed a decline in Hb, and RBC values after 28 days. The values of Red blood cells, haemoglobin, were decreased significantly ($P < 0.05$), depending on the Lead acetate dose. In group -1 compared with the group -2,3 and 4 and the toxicity of heavy metal decreased significantly and the WBCs values increased in experimental groups. Haematological parameters (RBC, Hb, WBC.) of fish of the experimental period of 15 to 28 days. The content of RBC, Hb and WBC of Rohu and Catla fishes showed (Table No.1 to 6 respectively)

Table 1: Hematological profile of experimental and control fishes exposed with Lead acetate

Parameters	Group-3	Group-4	Group-1			Group-2		
15th Day RBC 10^6/µl	TC	STDC	A1	B1	C1	A2	B2	C2
Pb-R-1	2.40±.003	2.38±.008	2.08±.008	1.97±.003	1.77±0.01	2.33±.003	2.19±.009	1.94±.017
Pb-C-1	2.40±.003	2.38±.008	1.89±.005	1.78±.018	1.64±.014	2.16±.012	1.96±.008	1.85±.020

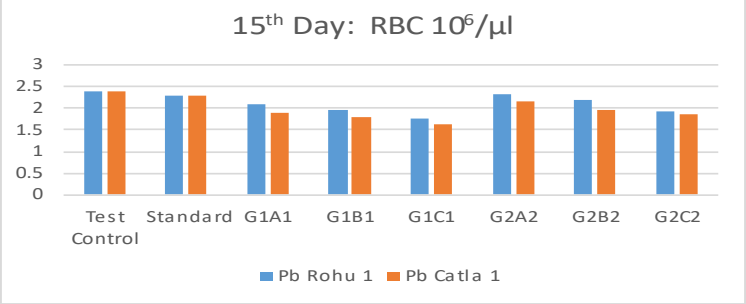


Table 2: Hematological profile of experimental and control fishes exposed with Lead acetate

Parameters	**Group-3**	**Group-4**	**Group-1**			**Group-2**		
28th Day RBC 10^6/µl	TC	STDC	A1	B1	C1	A2	B2	C2
Pb -R -2	2.40±.003	2.38±.008	1.63±.008	1.54±.01	1.82±.011	1.83±.008	1.74±.003	1.56±.013
Pb -C -2	2.40±.003	2.38±.008	1.45±.003	1.39±.005	1.36±.014	1.71±.003	1.57±.016	1.34±.008

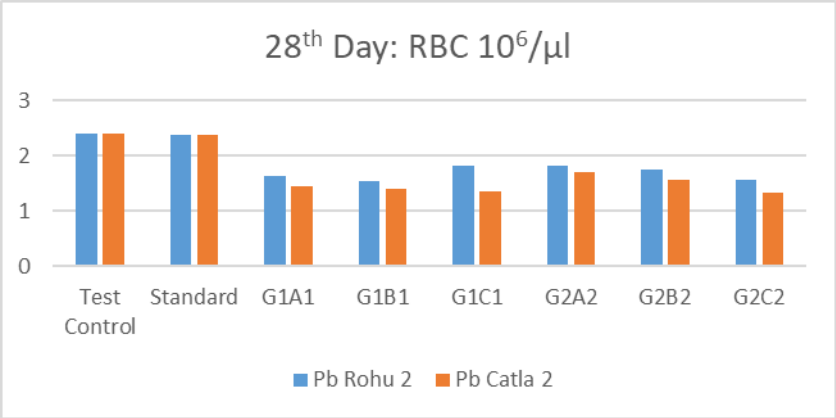


Table 3: Hematological profile of experimental and control fishes exposed with Lead acetate

15th Day WBC 10^3/µl	TC	STDC	A1	B1	C1	A2	B2	C2
Pb – R -1	6.42±.006	6.49±.045	7.01±.020	7.83±.034	9.01±.005	6.25±.026	7.02±.026	8.2±.230
Pb -C -1	19.24±.006	19.26±.038	27.05±.026	28.50±.037	31.17±.073	24.85±.028	25.04±.011	29.64±.023

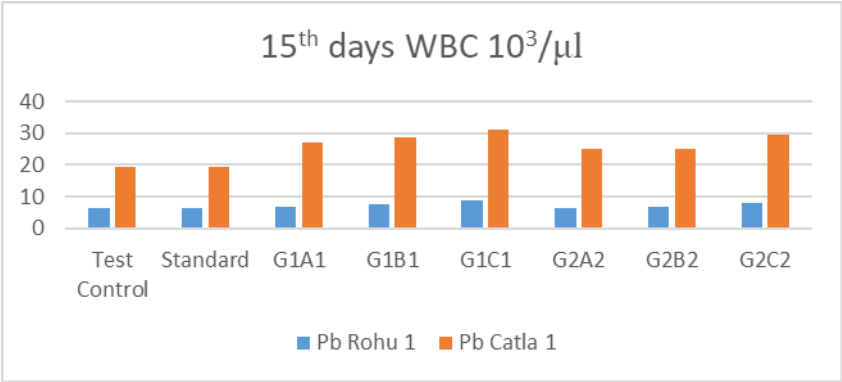


Table 4: Hematological profile of experimental and control fishes exposed with Lead acetate

Parameters	**Group-3**	**Group-4**	**Group-1**			**Group-2**		
28th Day WBC10^3/µl	TC	STDC	A1	B1	C1	A2	B2	C2
Pb -R -2	6.42±.006	6.49±.045	8.73±.015	9.63±.018	10.86±.014	7.92±.03	9.93±.033	10.15±.014
Pb -C -2	19.24±.006	19.26±.038	29.05±.008	32.03±.015	34.55±.021	24.84±.023	26.82±.01	32.36±.029

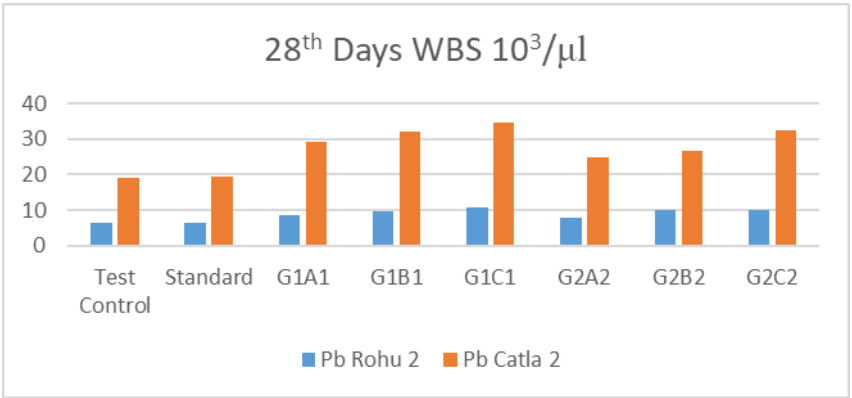


Table 5: Hematological profile of experimental and control fishes exposed with Lead acetate

Parameters	**Group-3**	**Group-4**	**Group-1**			**Group-2**		
15th Day Hb g/dl	TC	STDC	A1	B1	C1	A2	B2	C2
Pb -R -1	5.91±.003	5.87±.003	4.81±.020	4.47±.01	3.91±0.029	5.66±0.005	5.21±.01	4.83±.016
Pb -C -1	5.43±.005	5.41±.005	4.09±.016	3.78±.023	3.51±.016	4.93±.01	4.72±.020	4.21±.029

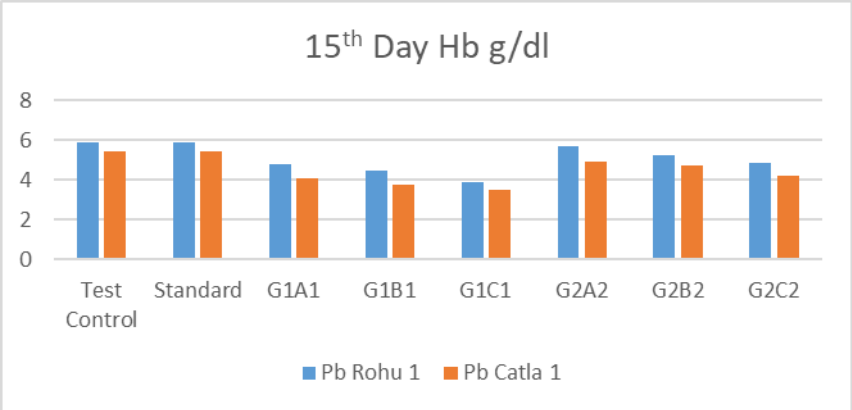
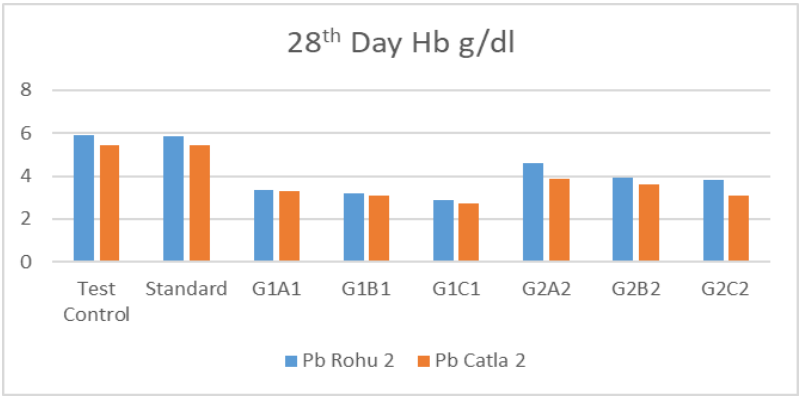


Table 6: Hematological profile of experimental and control fishes exposed with Lead acetate

Parameters	**Group-3**	**Group-4**	**Group-1**			**Group-2**		
28th Day Hb g/dl	TC	STDC	A1	B1	C1	A2	B2	C2
Pb -R -2	5.91±.003	5.87±.003	3.33±.026	3.21±.015	2.89±.026	4.59±.016	3.92±.008	3.82±.020
Pb -C -2	5.43±.005	5.43±.005	3.31±.01	3.11±.018	2.73±.016	3.89±.013	3.63±.015	3.11±0.009



Conclusion

In *Cassia tora* seeds the presence of organic compounds called glycosides improves the gastric secreating in the body for promoting digestion and can also helps by removing intensive heat from the liver, moisturizing intestine and contains chrysophanic acid -9-anthrone which is an important fungicide. As the dehydrated seeds of Cassia tora has good protein, it can be used as a full of protein feed for carps. Therefore, the present study suggest that the Heavy Metal Detoxification Diet acts as a detoxificant against heavy metal and are safe and have positive effects on the growth performance, antioxidant and hematological parameters of Rohu and Catla fishes.

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