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INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 13, Issue, 03, pp.16777-16781, March, 2021

ISSN: 0975-833X

DOI: https://doi.org/10.24941/ijcr.41001.03.2021

CASE REPORT **OPEN ACCESS**

A COMPARATIVE ANALYSIS OF PROTEIN AND GLUCOSE LEVEL IN INDIAN CATFISH TREATED WITH LEAD AND MERCURY

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ARTICLE INFO

Article History:
Received 25th December, 2020
Received in revised form
12th January, 2021
Accepted 15th February, 2021
Published online 30th March, 2021

Key Words:

Clarias batrachus,
Lead acetate,
Mercury chloride Toxicity,
Biochemical study

ABSTRACT

The present study aimed to assess the toxic effect of lead and mercury on aquatic sources as it indirectly or directly affects human beings. A freshwater Catfish *Clarias batrachus* was chosen as an ideal model for the experiment, as it can survive in unfavourable conditions. Sub lethal toxicity studies of lead and mercury on the Indian freshwater Catfish revealed significant changes in the biochemical constituents of the fish like total protein and glucose after 15 days of exposure to different lead acetate concentrations i.e. 0.2mg/l, 0.6mg/l and 1mg/l; the mercury chloride concentrations i.e. 0.0002mg/l, 0.002mg/l, and 0.02mg/l. Liver, kidney, gill and muscle tissues of control of treated fishes were taken for the protein estimation and blood samples of control and treated fish were considered for estimation of glucose. Depletion of protein was observed in various tissues of the treated fish as compared to the controlled fishes. The progressive elevation of plasma glucose level was observed in treated fish as compared to the controlled fishes in case of both lead and mercury treatment. The current findings suggest that the fish treated with lead have faced a serious metabolic crisis that also observed as nearly equivalent to the fish treated with a very low concentration of mercury.

Citation: Akansha Mishra and Bhaskar Behera. "A comparative analysis of protein and glucose level in Indian Catfish treated with lead and mercury.", *2021. International Journal of Current Research, 13, (03), 16777-16781.*

INTRODUCTION

The man made efforts to support the standard of living by rapid industrialization and urbanization which ultimately increase the level of pollutants in nature (Edwards, 1973; Hu, 2011; Paital *et al.*, 2016). Now a days intensive farming practices have become essential to produce enough food for increasing population by using more inorganic fertilizers, pestisides and herbisides which are highly toxic to our environment (Kumar, 2014; Tong, 2007; Begum, 2004). Among all kind of pollutions, the aquatic ecosystem pollution has become the greatest matter of concern in recent time. Specific effluents released by industries, minings etc. contains heavy metals, one of the major source of pollutants have become potential threats for the aquatic ecosystem (Manzano *et al.*, 2015; Singh *et al.*, 2019). The adverse effect of wastewater is not limited to aquatic animals but it also affects those that consume water from the polluted rivers or streams (Lu *et al.*, 2015; Chagas *et al.*, 2019).

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Lead as an industrial pollutant and immunotoxicant, has the likely to adverse affect human and animal health (Elarabany, 2019; Izah, 2016; Praveena, 2017). It induces a broad range of physiological, biochemical and neurological dysfunctions in human (Nordberg *et al.*, 2007; Alkahemal-Balawi *et al.*, 2011). Whereas mercury adversely effects various metabolic processes of the human body (Petering, 1976; Pandey, 2014; Mishra *et al.*, 2019). Minamata disease by mercury, itai-itai by cadmium,lead leprosy, metal fume, arsenic poisoning fever are few examples of effect of metal toxicity (Abbas, 2007; Kumar, 2016; Pichhode, 2020). Both the heavy metals can cause neurological, immunological, reproductive, biochemical and histopathological disorders in animals (Abou-Donia *et al.*, 1988; Makwana, 2020; Pandit, 2018). Consequently, fish are one of the direct victims of numerous detrimental effects by the heavy metal pollution of the aquatic environment (Carbonell, 1998; Tamele, 2020). Sometimes a large number of dead fish or unusual activities in fish are seen in aquatic sourses connected with sewages (Hellou, 2011; Little, 1990). The walking Catfish (*Clarias batrachus*) occupies an important status for its economy and high nutritive value in India (8). It can survive in unfavourable conditions with less amount of water for days.

Thus, *Clarias batrachus* can be considered a good model to study the effects of lead and Mercury in an aquatic ecosystem.

METHODS

The healthy and active specimen of fish having average body length 9.5±0.5cm and average body weight (12±0.5g) procured from a commercial hatchery, Balaramgadi, Balasore. Selected specimen were treated with 0.05% $KMnO_4$ solution for 2 min exposure to avoid any dermal infection and acclimatized to the laboratory conditions for 30 days. Further they were placed in large plastic tubs of 25 litres capacity with running tap water and fed with commercial fish food once in daily. During study 12hr light and 12hr dark photoperiod was maintained for acclimatization and exposed to test chemicals. The healthy acclimated fish having equal sized were chosen and sorted into 4 groups for every test compound contains 15 fishes each and exposed to different sub lethal concentration of lead (0.2mg/l, 0.6mg/l, 1.0mg/l along with a control group), acetate and mercury chloride (0.0002mg/l,0.002mg/l,0.02mg/l along with a control group) for 14 days. The water of each experimental tub was changed at regular interval and aerated with mechanical air pump. The behaviour and condition of the studied fish were noted everyday during the exposure period. Before these the LC_{50} value for lead (1.25 mg/l) and mercury (0.04mg/l) exposed fish were carefully calculated.

Table 1. Physio-chemical parameters of water during the experiential period

Water quality parameters	Calculated value
Temperature	29±2°C
pH value	7.0±0.2
Dissolved oxygen	6.4±0.5(mg /L)
Total hardness as $CaCo_3$	41±8(mg/L)
Total suspended solid	43±6(mg /L)

SAMPLING

Healthy fingerlings from both the treatment and control groups were sacrificed for different biochemical assays. Collection of blood sample was done according to the method of Kumari et. al (2010). Blood also collected from the heart then transferred to clean dry centrifuge tubes afterwards left for 2 hr for clotting and centrifuged at 3000 rpm for 15 min, followed by serum separation and storage in deep freeze(-20^{o}c) till biochemical analysis. Subsequently tissues were quickly removed in the following order: gills, liver, kidney and muscle cleaned from any extraneous materials and immediately perused with saline water. The tissues were homogenized in phosphate buffer solution (0.1 M, pH 7.4) and centrifuged at 1500 rpm for 20 min. The supernatant was stored in deep freeze until the biochemical analysis carried out.

BIOCHEMICAL ASSAY: Quantitative estimation of total protein from sample was done by the method of Lowry et al; (1951) using Bovine Serum Albumin (BSA) as a standard solution. Further blood glucose was estimated by following Folin and Wu (1919) method.

Protein content: For plotting the standard curve, a set of standard solution was run from 0.1 to 1.0 concentration in ml. The standard solutions were taken in a series of sterilized test tubes. The volume in each test tube was made up to 1 ml with added distilled water. Subsequently, 5 ml of Lowery reagent was added, mixed and then allowed to stand for 10 min at room temperature. Again 0.5 ml of Folin-ciocalteau phenol reagent was added to each tube and shaken well to mix properly. Consequently the developed blue colour was read at 660 nm wavelength after 20 min against a reagent blank in the UV/VIS spectrophotometer (Model: Systonic 117). The standard graph was drawn by plotting the concentration of standard solution on the Y-axis and the optical density on the X -axis.

For the quantitative estimation of protein, the studied tissue is homogenized with 0.1 ml Tris-HCL buffer solution and centrifuged at 10,000 rpm for 10 min. Further 0.1 ml of the serum was taken and made up to a final volume of 1 ml by adding double distilled water. The amount of protein present in 0.1 ml of the sample was calculated by referring to the standard curve obtained.

Glucose content: The blood sample was collected from the heart of the experimental animal after deproteinisation process. Three Folin Wu tubes were marked as A, B and C for analysis, standard and blank correspondingly. Moreover 2 ml of protein free filtrate was taken in tube A, 2 ml of standard glucose solution in tube B and 2 ml double distilled water in tube C. In each test tube (A,B,C) 2 ml of alkaline copper reagent was added. The content of each tube was mixed well by tapping gently between palms. The tubes were kept in boiling water bath for 8 min. After water bath the tubes were removed and immediately cooled under running tap water for 1 min. Phosphomolybdic acid (2ml) was added without delay to each sample loaded tube. The contents were tapped between palms until the effervescence stopped. The tubes were heated in water bath for 1 min to stabilized the colour. It was cooled and made up to the mark with water. The contents were mixed well by inverting the tubes. The optical density was read at 680 nm wavelength.

RESULTS

Total protein content (mg/ml) in the control fish group and exposed group containing different sub-lethal concentration of lead acetate and mercury chloride were estimated. Protein content in the liver, kidney, gill and muscle of the control and the lead treated fish samples are shown in Table 2. Protein content (mg/ml) in the muscle, kidney, liver, and gill of the control and mercury treated fish are shown in Table 3. Plasma glucose level (mg/l) in normal and lead treated fish are shown in Table-4 Plasma glucose level (mg/dl) in normal and mercury treated fish are shown in Table-5.

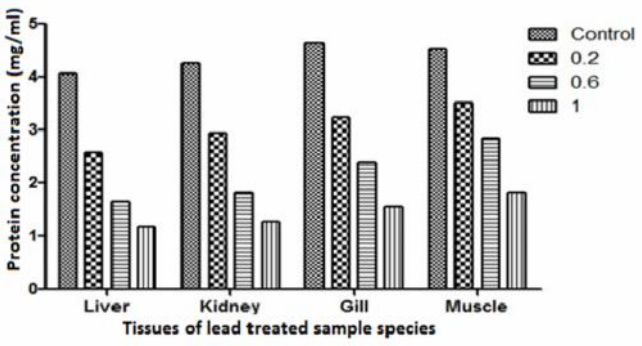

Figure 1. Shows depletion of protein in selected organs of lead treated fish

Table 2.

Tissues	Protein concentration (mg/ml)			
	Concentration of Lead acetate 0.0 mg/l	Concentration of Lead acetate 0.2 mg/l	Concentration of Lead acetate 0.6 mg/l	Concentration of Lead acetate 1.0 mg/l
	Control set	Treatment set		
Liver	4.065	2.58	1.645	1.18
Muscle	4.25	2.95	1.835	1.275
Kidney	4.625	3.23	2.39	1.555
Gill	4.53	3.51	2.855	1.835

Table 3.

Tissues	Protein concentration (mg/ml)			
	Concentration of mercury 0.0 mg/l	Concentration of mercury chloride 0.0002 mg/l	Concentration of mercury chloride 0.002 mg/l	Concentration of mercury chloride 0.02 mg/l
	C control set	Treatment set		
Liver	4.81	3.23	1.925	0.81
Muscle	4.065	2.855	1.555	1.09
Kidney	3.975	1.925	1.74	1.37
Gill	3.975	3.23	2.3	0.81

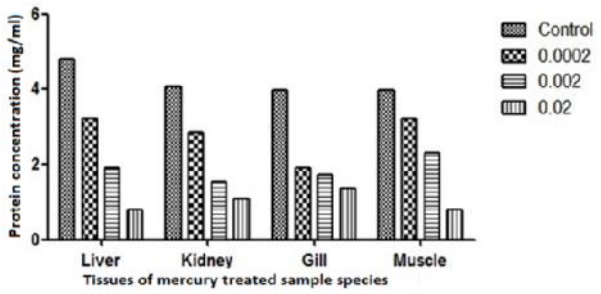

Figure 2. Shows depletion of protein in selected organs of mercury treated fish

Table 4.

Fish	Concentration of lead acetate (mg/l)			
	Control	Treatment set		
		0.2	0.6	1.0
Plasma glucose (mg/dl)	21.96	21.73	34.63	48.5

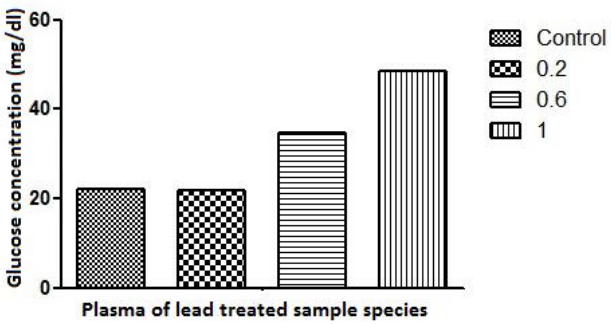

Figure 3. Shows elevation of plasma glucose in lead treated fish

DISCUSSION

Fishes are the excellent models for monitoring environmental contamination in aquatic ecosystem (Choudhury, 2018).

Table 5.

Fish	Concentration of Mercury chloride (mg/l)			
	Control	Treatment set		
		0.2	0.6	1.0
Plasma glucose mg/dl	20.33	21.36	38.41	45.12

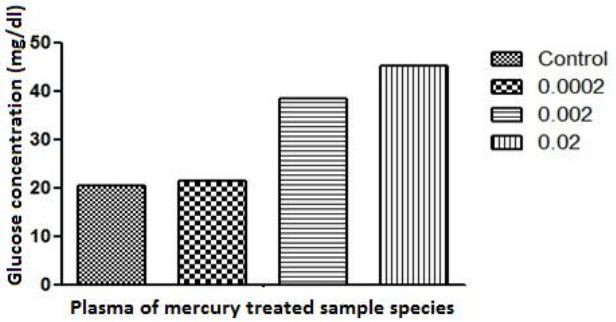

Figure 3. Shows elevation of plasma glucose in mercury treated fish

Exposure to different concentrations of lead and mercury produced severe biochemical abnormalities in fish body that were ultimately reflect through lowering in fish growth and their activeness. It also associated with biochemical changes in Catfish indicating altered metabolism. In the present study fish were exposed to sublethal concentrations of both lead i.e 0.2, 0.6 and 1.0 mg/l and mercury i.e 0.0002,0.002 and 0.02 mg/l. A single fish death was not observed throughout the entire experimental period. Gradually lowering of protein level observed in each collected tissue sample (liver, kidney, gill and muscle) of both lead and mercury treated fish group. The reduction of total protein might be attributed to the destruction or necrosis of cells and consequent to impairment in protein synthetic machinery (Mehra, 1980). Current study indicate that the highest decrease of protein is occurred in liver tissue as compared to other tissues in both lead and mercury treated fish groups.

Whereas the lowest protein depletion is observed in muscle tissues of lead treated fish group while in mercury treated fish group having lowest protein depletion within gill tissue. Among the biochemical profiles, plasma glucose has been extensively used as a sensitive indicator of environmental stress in fish (Nemcsok, 1982). Elevation of plasma glucose level in blood is observed in our present study for both lead and mercury treated fish group. The significant enhancement in plasma glucose level during the treatment may due to gluconeogenesis to provide energy for the increased metabolic demands imposed by the heavy metals (lead and mercury). In addition, the hypothalamo-pituitary interrenal axis is stimulated by stressors and elevated blood levels of cortisol which in turn leads to lipolysis, glycogenolysis and gluconeogenesis to provide energy under the stress conditions (Hontela, 1993).

CONCLUSION

It also noted that sub-lethal toxicity studies of lead acetate and mercury chloride on the Indian fresh water Catfish revealed significant changes in the biochemical constituents like total protein and glucose. Furthermore the food conversion efficiency of studied Catfish was also reduced after the exposure to lead and mercury. The experimental results suggest that the fish treated with lead have faced a serious metabolic crisis. It might be found to be nearly equivalent to the fishes treated with a very low concentration of mercury which clearly indicates that the discharge of effluents containing lead and mercury in to the water bodies may be a serious threat to aquatic flora and fauna as well as humans.

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