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Barium Chloride impairs physiology and brain glutamate in *Cirrhinus mrigala* during a short period of interaction

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ABSTRACT

The present study was performed to establish the toxicological impact of barium chloride ($BaCl_2$) on freshwater fish *Cirrhinus mrigala*. In this direction we have investigated the ecological impact of $BaCl_2$ on *C mrigala*, a freshwater fish widely consumed. Biochemical, neurochemical, and physiological variations were carried out in the present study and LC_{50} of $BaCl_2$ was found to be 12 ppm, observed for 24hrs. Further $1/10^{th}$ of the LC_{50} concentration of $BaCl_2$ (1.2 ppm) was selected for acute investigation for 96hrs. The results exhibited an increase in serum lactate dehydrogenase (LDH), aspartate transaminase (AST), and alanine transaminase (ALT) levels compared to control groups. Studies revealed alterations in oxidative stress markers with a notable lowering in the serum superoxide dismutase (SOD), and catalase (CAT) levels, and an increase in malondialdehyde (MDA). The studies reported an elevation in brain glutamate concentrations indicating brain tissue damage. The study highlights the potential toxicological impact of $BaCl_2$ on freshwater fish.

INTRODUCTION

The presence of xenobiotics and chemicals in the ecosystem due to their multipurpose applications and their let-outs lead to contamination of diverse ecosystems **(Rashmi *et al.*, 2019; Shyamala *et al.*, 2020)**. Most of these chemicals, in the form of heavy metals and their derivatives, are not degradable and have the potential to bind to endogenous molecules as they enter the biological system, and hence, may lead to unfavorable toxic effects **(Amdur *et al.*, 1991)**. The presence of these metals in the living system can lead to unwanted changes in a cell that may be cascaded to the tissue or the organ **(Bernet *et al.*, 1991)**. Thus, a scenario of these xenobiotics to accumulate in fish

and impair the food chain is created. Human beings, who consume fish products as food source, are also part of the food chain, thus generating a cause of concern in the public health (**Di Giulio & Hinton, 2008**). The influence of chemicals and their derivatives in the tissues of aquatic animals, like fish, depends on several parameters like the immediacy of the pollutants to animals, metabolic activity and membrane permeability, and climate **(Chen & Folt, 2000)**.

Barium has been reported to cause toxicity due to the high dose exposure **(Soudani *et al.*, 2011),** in addition, soluble salts of barium have been proved to be harmful to animals and plants **(Purdey *et al.*, 2004)**. Barium contamination could be related to firework, paper, textile, and other industries. Deleterious effects may occur due to the absorption of soluble barium compounds such as; nitrates and chlorides which are toxic to humans **(Ananda *et al.*, 2013)**. Following human ingestion, barium is reported to be toxic and extensively distributed in the heart, skeletal muscle, kidney, blood, and liver **(Mc Cauley & Washington, 1993)**. Thus we used freshwater fish *Cirrhinus mrigala* consumed extensively to assess the acute toxicity of barium chloride.

LC_{50} indicates the degree of resistance of a living system to xenobiotics **(Reda *et al.*, 2010)**. Physiological alterations in fish exposed to these xenobiotics give the precise level of damage. Toxicity stress causes oxidative damage by the generation of free radicals (ROS) with the exhaustion of natural antioxidants. Measurement of oxidative stress markers, such as; superoxide dismutase, catalase, and, lipid peroxidation, indicates the physiological variations due to toxicity. Changes in neurotransmitter glutamate content were assessed, because neurons are vulnerable to free radicals, resulting in modification of glutamate levels, and thus, resulting in neurodegenerative disorder **(Li *et al.*, 2010)**. In this direction, we evaluated the physiological and neurochemical (glutamate) disturbances caused by barium chloride in freshwater fish *C mrigala* in a short period exposure of 96 hrs.

MATERIALS AND METHODS

Animals and Treatments

Freshwater carp, *C mrigala* (6.6±0.8 cm of body length and 5.6±1.5g of weight) were obtained from a fish farm. Water quality was noted to be 30.8±2.3 $CaCo_3$mg/L of hardness and pH of 7.2±0.5, at a temperature of 23±1ºC. For experiments, fishes were distributed into three groups which were raised in 100L aquarium with continuous aeration.

Barium chloride ($BaCl_2$) sample preparation

12g/L of $BaCl_2$ solution was prepared in double-distilled water. The groups of fishes were treated with $BaCl_2$ for short duration of 96 hrs (4 days). The dosage of sample 1.2mg/L was based on $1/10^{th}$ of LC_{50}concentration (12mg/L).Water was changed daily and a freshly prepared solution of $BaCl_2$ was added. The control group of 50 fishes was housed with comparable conditions to the treated group. Feeding was inhibited 24 hrs before sacrifice to provide postprandial variations. Control and treated fishes (1.2 mg/L) were taken for further investigation. Zero mortality was observed during the entire 96 hrs of exposure to $BaCl_2$.

Sample collection

The cardiac puncture was made to draw the fishes blood. Samples, for physiological investigation, were transferred to EDTA vials following centrifugation at

9392g at 4ºC for 20 min. The brain tissue, for analysis of glutamate, was dissected and cryopreserved. During analysis the brain tissue was homogenized with 8 volumes of cold Tris buffer saline (10mM Tris-HCl, 10mM sucrose, 0.7% NaCl, 0.1mM EDTA of pH 7.2) at 4ºC. The mixture was centrifuged at 3000 rpm at 4ºC for 12 min, and the supernatant was taken for experiment.

Physiological parameter analysis

Blood glucose level was measured by preparing a reaction mixture of 0.1ml of plasma, 4ml of o-toluidine **(Cooper, 1970)**, and mixed finally incubated for 10 min on a boiling water bath. The samples were cooled and the absorbance was measured at 630 nm in a UV spectrophotometer. The glucose content was expressed as mg/dL. Plasma protein levels were assessed by the method of **Lowry et al. (1951)**. A100 µl of plasma, 900 µl of double distilled water and, Lowry reagent 4 ml was mixed well and incubated for 10 min at room temperature. A 0.5ml of FC reagent was added to this mixture and incubated for 20 min at room temperature. The sample was then read at 720 nm and expressed in µg/ml.

Serum transaminases analysis

Serum L aspartate aminotransferase and L-alanine aminotransferase activities were investigated at 37^{o} C following a colorimetric protocol of **Reitman and Franckel (1951),** and enzyme activity was measured as IU/L. Lactate dehydrogenase was evaluated by the procedure formulated by **Anon (1984)** and was expressed as IU/L.

Oxidative stress markers and glutamate analysis

Superoxide dismutase (SOD) was assessed **(Das *et al.*, 2000)** through the inhibition of superoxide led nitrite generation from hydroxylamine hydrochloride. The absorbance was calculated at 540nm and expressed as U/mg of protein. Catalase activity was analyzed depending on the generation of stable hydrogen peroxide with ammonium molybdate, read at 405 nm and expressed as U/mg **(Goth, 1991)**. Lipid peroxidation was measured with malondialdehyde (MDA) as standard **(Buege & Aust, 1978).** The reaction of the mixture of sample and TCA-TBA HCl reagent was boiled for 10min, cooled, and then centrifuged at 10000g. The supernatant was used for quantification at 535nm and expressed as nmol/mg. Glutamate levels were identified by multiple developments of paper chromatography **(Raju *et al.*, 2004).** The supernatant was evaporated at 70ºC and mixed with 100 ml double distilled water. The sample, with 2 mM glutamate standard solution, was spotted on Whatman no.1 chromatography paper. Then, it was allowed to develop on the mobile phase (Butanol: acetic acid: water 12:3:5 v/v). The chromatogram developed again, the papers were dried and sprayed with ninhydrin and incubated at 100ºC for 4 min. The bands which reveal glutamate corresponding to the standard was cut and eluted in 75% ethanol with 0.005% $CuSO_4$. The absorbance was read against the blank at 515nm in a spectrophotometer. The concentration was expressed as µmol/g of glutamate.

Statistical analysis

The experimental results were statistically evaluated and represented as Mean ± SE. For all experiments, the degree of statistical significance was set for $P<0.05$. The significance was determined by Student`s *t*-test using MS-Excel.

RESULTS

This study investigated the impact of $BaCl_2$ exposure on the physiological changes and glutamate contents in freshwater carp *C. mrigala*, by examining the physiological variables, oxidative stress and glutamate levels. The fish were distributed as treated with (1.2ppm of $BaCl_2$), while control groups were set for comparison, and all were under supervision for 24, 48, 72, and 96 hrs. The blood assessment of $BaCl_2$, treated with *C. mrigala*, reported a remarkable rise in glucose levels (Table 1) throughout the 4 days of the experiment, but, a major difference of 4.88±0.22/27.63±1.25 was observed on the 4th day (96 hrs). The decline in plasma protein level (table 1) was significantly (P<0.05) found in exposed groups (1.2 ppm of $BaCl_2$) on the 4th day (15.40±1.61/9.77±0.56) in comparison to the control groups. There was a distinct elevation in serum AST and ALT levels in exposed group, in all four days, compared to the control groups. Table 1 suggests the elevated activity of LDH in the treatment of *C mrigala* fish with $BaCl_2$ (1.2 ppm). Figure 1 reports the rise in brain glutamate concentration in treated fish (12.07±0.13) in comparison with the control group (10.3±0.22) on the 4th day.

Results in table 2 reveal a decline in SOD and CAT variables in the treated fish. The LPO evaluation in *C mrigala* exposed to 1.2ppm of $BaCl_2$, propose that liver may be the affected organ, as there is notable amplification in MDA levels.

Table 1: Changes in the physiological parameters of *C mrigala* exposed to $BaCl_2$ (1.2 ppm)

Biochemical variables	Exposure duration (in h) 1.2 ppm							
	24		48		72		96	
	C	E	C	E	C	E	C	E
Glucose (mmol/L)	3.26 ±0.15	7.45 ±0.33*	4.53 ±0.67	23.11 ±1.18*	4.72 ±1.04	26.17 ±0.29*	4.88 ±0.22	27.63 ±1.25*
Protein (g/L)	14.53 ±0.36	21.42 ±0.55*	14.63 ±0.27	19.12 ±0.34	13.78 ±0.58	14.75 ±1.06*	15.40 ±1.61	9.77 ±0.56*
LDH (IU/L)	3.45 ±0.58	7.46 ±0.33*	4.03 ±0.27	7.07 ±1.13*	3.92 ±0.66	6.88 ±1.05*	4.14 ±0.31	7.14 ±1.22*
AST (IU/L)	15.19 ±0.45	20.54 ±1.18*	15.54 ±1.15	20.47 ±0.83*	14.97 ±0.36	21.59 ±0.95*	15.63 ±0.28	22.39 ±1.18*
ALT (IU/L)	23.62 ±1.19	25.95 ±0.35*	23.08 ±1.32	27.17 ±1.05*	21.45 ±0.28	27.85 ±1.37*	23.26± 0.51	28.53 ±1.22*

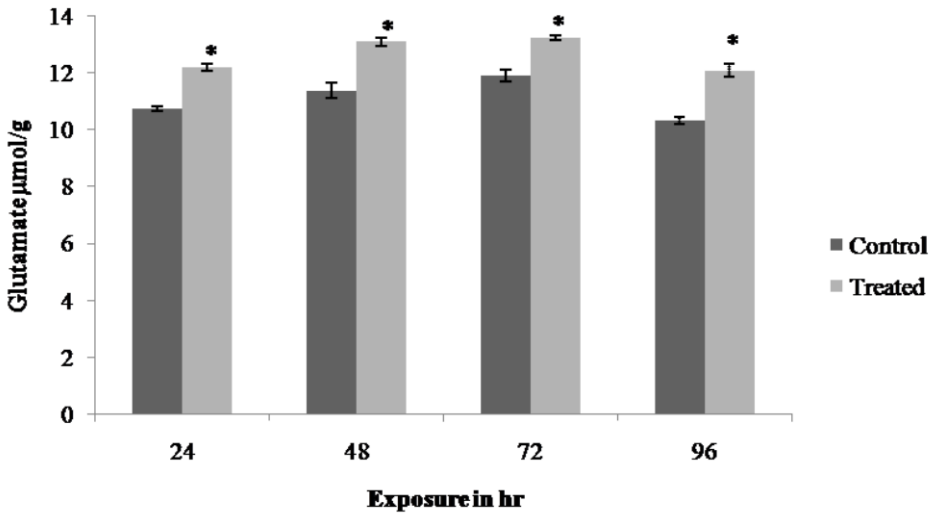
All values are expressed as mean± SE of three individual samples,* P < 0.05 is significant.

Table 2: Variations in the ROS markers of *C mrigala* exposed to barium chloride (1.2 ppm)

ROS variables	Exposure duration (in h) 1.2 ppm							
	24		48		72		96	
	C	E	C	E	C	E	C	E
SOD (U/mL protein)	5.32 ±0.71	3.94* ±0.19	5.57 ±0.07	4.12* ±0.26	4.98 ±0.37	4.02* ±022	5.12 ±0.44	3.97 ±0.34
CAT (µmol/ml protein/min)	2.54 ±0.04	1.96 ±0.35*	2.99 ±0.12	2.01 ±0.18*	2.43 ±0.14	1.68 ±0.29*	2.27 ±0.77	1.84 ±0.48*
nmol of MDA/mg protein)	4.38 ±0.54	4.88 ±0.63*	4.77 ±0.66	5.09 ±0.44	4.92 ±0.15	5.14 ±0.92*	3.98 ±0.21	4.97 ±0.56*

All values are expressed as mean± SE of three individual samples,* $P < 0.05$ is significant.

Fig 1: Alterations in the brain glutamate level of *C. mrigala* exposed to $BaCl_2$ (1.2 ppm) upon short term treatment



All values are expressed as mean± SE of three individual samples,* $P < 0.05$ is significant

DISCUSSION

The physiological modifications during interaction of freshwater fish C *mrigala* and 1.2ppm of $BaCl_2$ was recorded by studying different variables. There was a considerable rise in glucose levels in treated fish. The outcome can be credited to hyperglycemia due to stress. Comparable findings are reported, which are attributed to the changes in carbohydrate metabolism, owing to glycogenolysis in fish. Elevation in glucose upon metal exposure has been reported, because of glucose synthesis from amino acids of extra hepatic tissue **(Almeida, 2001; Banaee *et al.*, 2019)**. Other reports

correlated raise in plasma glucose contents to the release of glucocorticoids and catecholamines from adrenal tissues of fish under toxic induced stress **(Klaper, 2010)**. The protein levels progressively declined in the treated fish as period of exposure increased. The reduced protein content is associated with binding of metal to blood and tissue proteins, resulting in tissue damage and oxidative stress. This process changes the physicochemical characteristics which causes change in protein structure conformation (**Chen *et al.*, 2011**), thus may be the basis of reduction in protein concentration in the present study. The toxicity by metals is the rationale behind declined protein levels as it inhibits transcription and translational processes.

The abnormal presence of transaminases; like aspartate transaminase (AST/GOT) and alanine transaminase (ALT/GPT) in the blood, is a sign of tissue damage. The detection of these biomarkers is usually related to inhibition of enzyme in metabolism due to exertion of stress. In the current study there was a notable elevation in both ALT/AST levels in treated fish. These findings are due to tissue and organ injury caused by contact to toxicants **(Abhijith *et al.*, 2016)**. These observations indicate the role of transaminases as major biomarkers during exposure to xenobiotics. AST and ALT are markers in liver function tests, as AST is synthesized by liver hepatocyte and typically found in liver and heart, while ALT, basically, is present in liver and kidney. Consequently, increased activity of these enzymes has been reported in fish exposed to xenobiotics **(Rao, 2006)**. The raise in LDH can be ascribed to rapid glycolysis due to metabolic stress. Additionally, the onset of anoxia is also a cause for higher LDH activity. The anaerobic condition caused due to metal toxicity is reported to increase LDH activity **(Min *et al.*, 2008).**

Figure (1) displays higher brain glutamate contents in treated fish compared to the control. This may be integrated with exotoxicity and undue generation of glutamate, and that may result in death and damage of nerve cells. The high glutamate levels changes the brain physicochemical environment, leading to the activation of the glutamate receptors that allows high concentration of calcium ions to permeate the cell **(Manev *et al.*, 1989)**. Generation of free radicals (ROS) is the reason for oxidative stress, thus disturbing the biological processes by upsetting homeostasis **(Chandra *et al.*, 2017)**. This causes imbalance in equilibrium of the detoxification mechanism of reactive oxygen species through antioxidants **(Manke *et al.*, 2013)**. To scavenge the excessive generation of free radicals, cells adopt the mechanism of enzymatic and non-enzymatic responses **(Chandra & Sukumaran, 2020)**. Xenobiotics cause free radical generation during mitochondrial respiration and activate NADPH-like enzymes. In this study, superoxide dismutase and catalase activity decreased periodically with exposure to barium chloride. Enzymes; like superoxide dismutase (SOD) and catalase (CAT), play chief role in scavenging free radical produced during stress to the metabolic and physiological processes. Superoxide dismutase has been shown to be the immediate response to oxidative stress in biological system **(Puneeth & Chandra, 2020)**. This is because of the O_2 generation and its conversion to H_2O_2, which leads to oxidation of cysteine in the antioxidant enzyme, thus confirming $BaCl_2$ toxicity.

Lipid peroxidation (LPO) is the primary cause in the damage of cell structure and function, because of excessive production of free radicals (**Huang *et al.*, 2003)**. The lipid peroxidation mechanism is understood by measuring the malondialdehyde (MDA) levels, which is one of the end products of lipid breakdown due to peroxidation. The current

result suggests that the existing cellular defense mechanism was not competent to avert the oxidative damage **(Chandra *et al.*, 2020; Chandra & Sukumaran, 2020; Jagadeep *et al*., 2020)**.

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