

Magnesium chloride impairs physio-biochemical and neurochemical responses in *Cirrhinus mrigala* (Hamilton, 1822) upon short term exposure

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ABSTRACT

The present investigation was performed to establish the biochemical and physiological impact of Magnesium chloride (MgCl₂) on fresh water fish *Cirrhinus mrigala*. In this direction we have evaluated the biochemical and neurochemical impact of MgCl₂ on *C. mrigala*, a fresh water fish extensively consumed. Biochemical, neurochemical and physiological alterations were analyzed in the present study and LC₅₀ of MgCl₂ was found to be 192 ppm, observed for 24h. Further 1/10th of the LC₅₀ concentration of MgCl₂ (19.2 ppm) was chosen for short term examination at 96 h. The results demonstrate elevation in levels of serum lactate dehydrogenase (LDH), aspartate transaminase (AST) and alanine transaminase (ALT) levels compared to control groups. Studies revealed variations in oxidative stress markers with significant reduction in the serum super oxide dismutase (SOD) and catalase (CAT) levels, and increase in malondialdehyde (MDA). The study showed an increase in brain glutamate levels reflecting possible brain tissue damage. The above study highlights the potential biochemical impact of MgCl₂ on fresh water fish.

Keywords: Biochemical, *Cirrhinus mrigala*, Magnesium chloride, Neurotransmitter, Physiology.

Abbreviations: ALT: Alanine transaminase, AST: Aspartate transaminase, CAT: Catalase, LDH: Lactate dehydrogenase, MDA: malondialdehyde, ROS: Reactive oxygen species, SOD: Superoxide dismutase.

1. INTRODUCTION

Existence of xenobiotics, chemicals and their derivatives in the environment and ecosystem due to their agricultural and industrial applications are well documented. The majority of these chemicals in the form of heavy metals and their derivatives are not degradable and have the possibility of binding endogenous molecules as they enter the biological system may cause unfavorable toxic effects [1]. The existence of these metals in biological system can bring about undesirable modifications in a cell which may be circulated to the tissue or the organ [2]. The eventuality of these metals to accumulate in the aquatic ecosystem particularly fish, have detrimental impact on the food chain. Human populations who consume aquatic products as primary source of food are also part of the food chain, thus generating a cause of concern in public health [3]. The impact and bioaccumulation of metals and their derivatives in the organs of aquatic animals like fish depend on variety of factors like proximity of the animals from the pollutants, metabolic activity and membrane transport potential, climatic conditions [4, 5].

Magnesium chloride salts are ionic halides and highly soluble in water. They exist as various hydrates MgCl₂ (H₂O)_x. Anhydrous magnesium chloride is the primary precursor of magnesium metal produced in industrial scale. However hydrated magnesium chloride is the most readily accessible form. Magnesium chloride is extensively used in dust and erosion control, catalyst control, ice control, horticulture and gardening

etc. The above applications lead to environmental and biological presence and interference of magnesium chloride. Aquatic ecosystem being the most susceptible for accumulation of any pollutants, acts as a strong indicator of its biological and ecological interactions. Thus in this direction we adopted fresh water fish *Cirrhinus mrigala* consumed extensively for the measurement of acute toxicity of magnesium chloride to fresh water fish.

LC₅₀ reflects the the extent of resistance of an organism to metal and its derivatives [6]. Biochemical and physiological understanding of exposed fish gives the precise extent of damage caused by these metals. Toxic stress leads to oxidative damage by the production of ROS consequently with exhaustion of natural antioxidants.

Quantification of oxidative stress markers such as SOD, catalase and lipid peroxidation (LPO) demonstrates the biochemical variations due to toxicity. Alterations in brain neurotransmitter glutamate are assessed given that neurons are susceptible to ROS, ensuing in variations of neurotransmitter and causing neurodegenerative disorders due to oxidative damage [7].

Thus in the current research we evaluated the biochemical, neurochemical (glutamate) and oxidative stress imparted to fresh water fish *C. mrigala* upon short term exposure (96 h) to magnesium chloride.

2. MATERIALS AND METHODS

2.1. Animals and treatments.

Indian fresh water juvenile carp, *C mrigala* (6.4 ± 0.8 cm of body length and 5.5 ± 1.3g of weight) were obtained from fish

farm, Karnataka, India. The fishes were acclimatized in flow through system in dechlorinated water for 15 days. Water quality was determined and reported to be 32.5 ± 2.2 calcium

carbonate mg/L of hardness and pH of 7.2 ± 0.3 , at a constant temperature of $23 \pm 1^\circ\text{C}$ with a light dark cycle of 12:12 hrs. During this period the fishes were fed with commercial fish food. For experiments, fishes were segmented into two groups which were raised in 100 L aquarium with continuous aeration.

2.2. Magnesium chloride (MgCl_2) sample preparation and inoculation.

192 ppm of solution was prepared by dissolving MgCl_2 in double distilled water. The groups of fishes were exposed to MgCl_2 for short period of 96 h (acute toxicity). Concentrations of sample 19.2 ppm based on $1/10^{\text{th}}$ of LC_{50} concentration (192 ppm) were introduced into 100 L aquariums containing 50 fishes each. The fishes with similar size and weight were separated as groups. Every 24 h the water was changed and freshly prepared solution of MgCl_2 was introduced to the aquariums containing fishes. Control group of 50 fishes was also maintained under the similar physical conditions like the treated group fishes. Food was withdrawn 24 h before sacrifice to circumvent postprandial variations. Control and treated fishes (19.2 ppm) were taken for further investigation. Nil mortality was observed during the entire 96 h of exposure to magnesium chloride.

2.3. Sample collection.

Blood from the fishes was drawn through cardiac puncture. The blood drawing syringes were pre-coated with heparin, an anticoagulant. The samples for biochemical evaluation were immediately transferred to EDTA vials and centrifuged at 9392 g for 20 min at 4°C . The brain tissue sample for estimation of neurotransmitter (glutamate) was immediately dissected and placed in ice. The brain tissue during the evaluation was homogenized with 8 volumes of cold Tris buffer saline (10 mM Tris-HCl, 10 mM sucrose, 0.7% NaCl, 0.1 mM EDTA of pH 7.2 at 4°C). The homogenate was then centrifuged at 3000 rpm for 12 min at 4°C , and the supernatant was retained for further evaluation.

2.4. Biochemical indices analysis.

Blood glucose level was determined by preparing a reaction mixture of 0.1 ml of plasma, 4 ml of o-toluidine [8] which was later mixed and incubated for 10 min on a boiling water bath. The samples were cooled and the absorbance was read at 630 nm in a UV spectrophotometer. The glucose concentration was expressed in mg/dl. Plasma protein concentration was measured by lowry's method [9] 100 μL of plasma, 900 μl of double

distilled water and 4 ml of lowry's reagent were mixed well and incubated for 10 min at room temperature, to this reaction mixture 0.5 ml of FC reagent was added and incubated for 20 min at room temperature. The sample was then read at 720 nm and expressed in $\mu\text{g/ml}$.

2.5. Analysis of serum transaminases.

Serum L aspartate aminotransferase (L-AST) and L-alanine aminotransferase (L-ALT) activities were reported by colorimetric method ([10] and enzyme activity was expressed as IU/L. Lactate dehydrogenase (LDH) was measured by the method developed by Anon (1984) and activity was expressed in IU/L.

2.6. Oxidative stress markers and glutamate analysis.

Superoxide dismutase (SOD) was measured [11] by the inhibition of superoxide led nitrite generation from hydroxylamine hydrochloride and the absorbance was determined at 540 nm and activity was expressed as U/mg of protein. Catalase activity was determined based on the formation of stable complex of hydrogen peroxide with ammonium molybdate and absorbance was measured at 405 nm and expressed as U/mg [12]. Lipid peroxidation was evaluated by taking malondialdehyde (MDA) as standard [13]. The reaction mixture of TCA-TBA HCl was boiled for 10 min, cooled and then centrifuged at 10000g and the supernatant was taken for measurement at 535 nm and expressed as nmol/mg. Glutamate levels were determined by multiple development of paper chromatography [14]. The supernatant was evaporated at 70°C and mixed with 100 ml double distilled water. 2 mM glutamate standard solution with the sample was spotted on Whatman no.1 chromatography paper which was then allowed to develop on the mobile phase (Butanol: acetic acid: water 12:3:5 v/v). The chromatogram was developed again, following which the papers were dried and sprayed with ninhydrin and incubated at 100°C for 4 min. The bands which reveal glutamate analogous to the standard are cut and eluted in 75% ethanol with 0.005% CuSO_4 . The absorbance is read against the blank at 515 nm in a spectrophotometer. The concentration is expressed as $\mu\text{mol/g}$ of glutamate.

2.7. Statistical analysis.

All data were statistically analyzed and represented as Mean \pm SE. In all experiments, the level of statistical significance was set for $P < 0.05$. The significance was calculated by Student's *t*-test using MS-Excel.

3. RESULTS

The present examination demonstrates the impact of MgCl_2 exposure on the biochemistry and physiology of fresh water carp *C. mrigala*, by understanding the biochemical, physiological and neurochemical variables. The fish were divided into treated (19.2 ppm of MgCl_2) and control groups and were studied for 24, 48, 72 and 96 h. The blood analysis of MgCl_2 treated *C. mrigala* suggests notable raise in glucose levels (table 1) throughout the 4 days of experiment, though significant difference of $6.23 \pm 0.22 / 23.77 \pm 0.93$ was recorded to be on the 4th day (96 h). The lowering in plasma protein level (table 1) was significantly ($P < 0.05$)

demonstrated in treated groups (19.2 ppm of MgCl_2) on the 4th day ($4.84 \pm 0.44 / 3.37 \pm 0.48$) in comparison to the control groups. In the present analysis there was a marked raise in both AST and ALT concentration of treated groups on all the four days in comparison to the control groups. Lactate dehydrogenase (LDH) is an isoenzyme playing a key task in glycolysis, which is also considered as important biomarkers of organ and tissue damage. Table 1 reports the increased activity of LDH on treatment of *C. mrigala* fish to MgCl_2 (19.2 ppm).

Table 1. Alterations in the biochemical variables of *C. mrigala* exposed to MgCl₂

Biochemical variables	Exposure duration (in h)							
	24		48		72		96	
	C	E	C	E	C	E	C	E
Glucose (mmol/L)	6.49±0.18	10.37±0.63*	6.13±0.34	16.19±0.39*	6.77±0.58	21.72±1.07*	6.23±0.22	23.77±0.93*
Protein (g/L)	5.31±0.51	11.48±1.07*	5.36±0.57	10.26±0.55	5.89±0.21	10.03±0.31*	4.84±0.44	3.37±0.48*
LDH (IU/L)	3.18±0.33	7.45±0.31*	3.61±0.3	7.18±0.46*	3.66±0.45	6.63±0.54*	3.89±0.13	7.49±0.96*
AST (IU/L)	11.53±1.21	15.12±0.99*	10.93±0.14	14.43±0.79*	11.37±0.31	16.53±0.35*	11.33±1.08	18.19±1.06*
ALT (IU/L)	13.57±0.63	17.81±1.23*	13.24±0.88	17.13±0.38*	13.05±0.38	18.28±0.75*	14.05±0.66	18.25±0.66*

All values are expressed as mean ± SE of three individual samples,* P< 0.05 is significant. (Control: C. Experimental: E)

Table 2. Alterations in the ROS variables of *C. mrigala* exposed to Magnesium chloride.

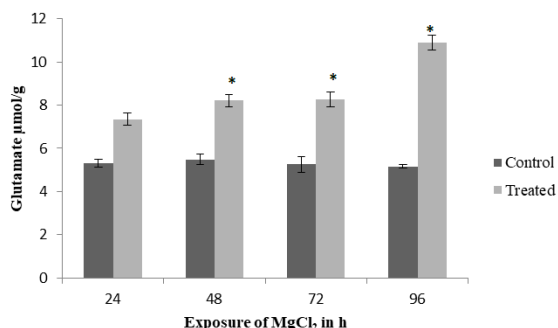
ROS variables	Exposure duration (in h)							
	24		48		72		96	
	C	E	C	E	C	E	C	E
SOD (U/mL protein)	7.58±0.94	5.32*±0.56	7.21±0.44	5.08*±0.41	7.36±0.49	5.17*±0.46	7.73±0.51	4.93±0.75
CAT (µmol/ml protein/min)	2.14±0.08	1.44±0.06*	2.93±0.19	1.85±0.37*	2.36±0.44	1.23±0.17*	2.91±0.49	1.03±0.12*
nmol of MDA/mg protein)	3.18±0.38	5.12±0.28*	3.46±0.16	5.06±0.17	3.92±0.57	5.19±0.43*	3.63±0.17	5.26±0.44*

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

Fig 1 reports the elevation in brain glutamate levels in treated fish (10.89 ± 0.35) when compared to the control groups (5.15 ± 0.08) on the 4th day. The results in table 2 demonstrate a decrease in SOD and CAT indices of the treated groups when compared to the control groups on all the days of sample treatment.

The LPO analysis in *C. mrigala* treated to 19.2 ppm of MgCl₂, suggest that liver might possibly be one of the affected organ, as there is a noticeable elevation in MDA levels in treated fish compared to the control fish (table 2).

The physio-biochemical and neurochemical modifications occurring during the exposure of fresh water fish *C.mrigala* with 19.2 ppm of MgCl₂ was recorded using various indices. There was a considerable elevation in glucose concentration of treated fish groups in comparison to the control fish. This observation can be attributed to the stimulation of stress resulting in hyperglycemia.



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

Figure 1. Alterations in the brain glutamate level of *C. mrigala* exposed to MgCl₂

Comparable results were reported which are ascribed to the variations in carbohydrate metabolism, on account of

glycogenolysis in fresh water fish. Rise in glucose levels during metals exposure has also been reported due to synthesis of glucose from extra hepatic tissue amino acids [15]. Several studies also associated increase in plasma glucose levels to discharge of glucocorticoids and catecholamines from adrenal tissues of fish during stress incidents [16].

The protein levels decreased (on 4th day) in the treated fish as duration of exposure was increased. The reduced protein levels are interrelated to metal binding to blood and tissue proteins culminating in tissue injury and oxidative stress. It also inflicts changes in physio-biochemical properties which are evident in protein structure conformational change [17], and this might also be the basis of a decline in protein concentration in the present investigation. Toxicity of metals might also be the rationale for declined protein levels as it inhibits transcription and translational processes.

The detection of transaminases like aspartate transaminase (AST/GOT) and alanine transaminase (ALT/GPT) in blood have been clinically employed to recognize any tissue damage. Presence of these biomarkers can be coupled to the enzyme inhibition in metabolic pathways upon exertion of stress. There was a notable elevation in both ALT/AST levels in treated fishes. These observations can be credited to the tissue and organ damage caused upon exposure to MgCl₂ and other xenobiotics [18]. Consequently, highlighting the potential role of transaminases as principal biomarkers during exposure to xenobiotics and ensuing in metabolic stress. AST and ALT are indicators in liver function tests, as AST is synthesized by liver hepatocyte and characteristically found in liver and heart, while ALT largely is present in liver and kidney. Subsequently higher activity of these enzymes has been reported in fish exposed to pesticides [19]. The raise in LDH can be attributed to amplified glycolysis upon metabolic stress. In addition, onset of anoxia is

also a sizeable reason for elevated LDH activity. Occurrence of anaerobic condition due to metal toxicity is also reported to rise in LDH activity [20].

The graph (fig 1) demonstrates the increase in brain glutamate levels in treated fish compared to control. This observation might be coupled with exotoxicity and overproduction of glutamate, which results in damage and death of nerve cells. The elevated glutamate concentration makes modifications in brain physicochemical milieu which activates the glutamate receptors by allowing soaring concentration of calcium ions to enter the cell [21].

Generation of reactive oxygen species (ROS) is the primary cause of oxidative stress, which further disturbs the biological processes by disconcerting homeostasis. This outcome is imbalance of equilibrium of the detoxification processes of ROS [22]. To scavenge the excessive generation of ROS, cells supply the various enzymatic and non-enzymatic responses. The pro-oxidant nature of metals causes ROS generation during mitochondrial respiration and thus activating NADPH-like enzymes. Enzymes like superoxide dismutase (SOD) and catalase

4. CONCLUSIONS

The result obtained in this investigation reveals that magnesium chloride interacted with *C. mrigala* for short duration (96 h), has both moderate and significant influence on biochemical, physiological and neurochemical indices. The study proposes that magnesium chloride at higher concentration (192 ppm) have displayed toxic effects on *C. mrigala*. Biochemical markers reflect the oxidative stress followed by oxidative damage through increase in lipid peroxidation, whereas inhibition of

5. REFERENCES

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(CAT) play a crucial role in scavenging ROS formed during alterations in metabolic and physiological processes. SOD has been reported to be the principle and instantaneous response to oxidative stress in a biological system [23]. This is due to the generation of O₂ and their conversion to H₂O₂, which may additionally lead to oxidation of cysteine in the antioxidant enzyme, thus reassuring MgCl₂ toxicity.

Lipid peroxidation (LPO) is the chief culprit in the disruption of cell structure and function due to unwarranted generation of reactive oxygen species [24, 25]. The lipid peroxidation process is determined by the measuring the malondialdehyde (MDA) concentration, which is one of the end products of breakdown of lipids due to peroxidation. The results suggest the existing cellular defense mechanism was not competent to ward off the oxidative damage.

All the above mentioned analysis suggests bioaccumulation of MgCl₂. The study proposes that the release of magnesium chloride to the aquatic environment may lead to detrimental effects on aquatic animals and the human population who are reliant on aquatic products as source of diet.

antioxidant enzymes SOD and catalase at higher concentration is also an indication of MgCl₂ interference. Notable increase in the neurotransmitter, glutamate levels also calls for more investigation for neurotoxicity effects of MgCl₂. Finally, though MgCl₂ exist in environment, any increase in the threshold levels of magnesium chloride levels may result in deleterious effect on both aquatic and terrestrial organisms.

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