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THE STUDIES ON PROTEIN CHANGES IN LIVER AND MUSCLE OF THE CATFISH, *CLARIUS GARIEPINUS* (BURCHELL, 1822) EXPOSED TO SUB-LETHAL CONCENTRATION OF MERCURIC CHLORIDE.

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ABSTRACT

The freshwater fishes are major and cheap sources of proteins for human beings. The nutritive value of a fish depends upon the quality and the quantity of proteins. Recently, there is a decrease in this nutritive value due the presence of various pollutants in the water bodies. The amount and the excellence of the nutritive substances in a fish tissue is inversely proportional to quantity of contaminants present in water body .Mercury has become a substantial pollutant of aquatic ecosystems due to its tendency of bio-magnification in the water bodies and the various toxic effects that it has on the fish and other aquatic animals. The effect of Mercuric chloride on the protein content of fresh water catfish, ***Clarius gariepinus*** **(Burchell, 1822)** *was evaluated. The 96-hours acute toxicity (LC50) of mercuric chloride is found to be 1.8mg/L. The test animals were exposed to 1/3rd and 2/3rd sub lethal concentrations of LC50 values of mercuric chloride as per recommendations for a period of 96hrs. After 24, 48, 72 and 96 hours of exposure; the protein levels were estimated in Muscles and Liver. The amount of protein decreases with an increase in concentrations of mercuric chloride and the time of exposure.*

Introduction

The freshwater fishes like *Labeo, Catla, and Clarius etc* are important and cheap sources of proteins for many people. The nutritive value of fishes depends upon quantity and quality of proteins present in Muscles of fishes. The amount and quality of nutritious materials is gradually degrading due to pollutants present in the water bodies. The quality and amount of proteins in fish tissue depends upon amount and type of pollutants present in water body. The major rivers of World are being polluted due to deposition and accumulation of

different polluting agents. These agents includes domestic and industrial discharges containing heavy metals, organic and inorganic chemicals, insecticides, pesticides, heat effluent, radioactive substances and domestic waste containing human faecal matter.

Mercury is found naturally in the environment in the metallic form and in different inorganic forms. Most of the mercury in the atmosphere is elemental mercury vapour and inorganic mercury, while most of the mercury in water, soil, plants and animals are inorganic and organic forms. Mercury has been used in many manufacturing industries such as plastics, chlorine, caustic soda and caustic potash and agricultural fungicides. These industries are responsible for releases of mercury and its other compounds into the water. Mercury is an extensive pollutant of considerable aquatic environment because of its tendency to bio-magnify in aquatic environment and its toxicity to fish and wildlife. Though mercury occurs naturally in the environment, but its concentrations have increased due to its use in the industrial processes [1]. The natural and anthropogenic sources including industrial and domestic sewage, leaching from landfills, shipping and dock activities and atmospheric deposits make entry of Heavy metals in aquatic ecosystem [2].

The inorganic form of mercury is methyl mercury (MeHg), it is highly toxic and quickly accumulates in the tissues of organisms [3] and [4]. In marine and freshwater sediments anaerobic sulphate-reducing bacteria synthesizes methyl mercury and aquatic fauna is subjected to contamination. The toxic effects of Mercury exposure are associated with its nervous system. Mercury is also concerned with immune suppression, endocrine disruption, and physical malformation [5] and [6].

Depletion of liver protein was observed at all concentrations and exposure periods which was highly significant ($p<0.01$) in fish exposed to 1 and 2 ppm of HgCl2 on 14th day, when compared to control. Depletion of protein content in the muscle of fish exposed to mercury chloride for 7 and 14 days in 0.5, 1.0 and 2.0 ppm concentrations were recorded. On 7th and 14^{th} day, highly significant ($p<0.01$) decrease in the muscle protein was observed in fish species exposed to 1 and 2 ppm concentrations of HgCl2, when compared to control [7]. The median lethal concentration (LC50 for 96 hrs) of cadmium in *L. calcarifer* was determined to be 6.08 ppm and for mercury 1.03 ppm. The acute toxicity of mercury and cadmium in *L. calcarifer* and stated that the fish was more susceptible to mercury, followed

by that cadmium. This study indicates that mercury was more toxic (1.03 ppm) metal than that of cadmium (6.08 ppm) [8].

The amount of protein in Muscles and Liver of *Clarius gariepinus* exposed to mercuric chloride decreased with the increase in the concentration and time of exposure to the heavy metals. The protein level decreased throughout the experimental period showing an increase of 0.6mg/litre and 1.2 mg/litre, at the end of 24, 48, 72 and 96 hours of treatment, respectively.

Material method

The freshwater fish *Clarius gariepinus* (Burchell, 1822) were separated from *Clarius batrachus* and collected by fisherman near Kumbharwada and were used for bioassay studies. The fishes were brought into the laboratory for the acclimatisation. The fish *Clarius gariepinus* (Burchell, 1822) were selected for experiments irrespective of their sexes. The size or length ranged from 30 cm to 34.5 cm and weight ranged from 251gm to 459gm. Fishes were acclimatized in glass tank in the laboratory for seven days as per the method in APHA [9]. The acclimatized fishes were used for the present experiments. Fishes were divided into different groups each containing ten fishes for the experiment. Mercuric chloride was weighed accurately as per requirement and dissolved in water before the transfer of fishes into the aquarium. Control group of fishes was maintained simultaneously.

The 96 h LC50 of mercuric chloride was determined following the graphical method of Krouwer and Monti. [10] and confirmed using regression analysis the LC50 values of Mercury chloride was 1.8 mg/litre. The test fishes were subjected to sub-lethal concentrations namely 1/3rd and 2/3rd of LC50 of Mercuric chloride as per suggestions for different exposure periods (24, 48, 72 and 96 hours) [11]. The equal number of fish was maintained in controls for similar duration of exposure [12]. In the scarified samples the Biochemical parameter such as protein was analysed by adopting method of Biuret reagent.

Result and Discussion

Table-1.

Effect of Mercuric chloride on protein content in Muscles of *Clarius gariepinus* (Burchell, 1822)

Concentraions ---in mg/litre	Exposure Time			
	24hrs	48hrs	72hrs	96hrs
Control	30.27769 ± 2.926554	29.44436 ± 2.545876	28.88877 ± 2.926559	29.16655 ± 3.004613
0.6 mg/lit.	28.61103*** ± 2.678761	25.55549* ± 5.091782	17.49995*** ± 5.204124	16.38876*** ± 3.938315
1.2 mg/lit.	29.44433* ± 4.589624	25.72189** ± 6.001095	16.94438*** ± 2.097168	14.99993*** ± 3.632368

Values expressed as -- --mg /gram of body weight,

± = Standard deviation of three observation.

* = Insignificant, ** = Significant at 5.0%, ***= Significant at 1 %,

ANOVA table was used for calculation.

Graph-1

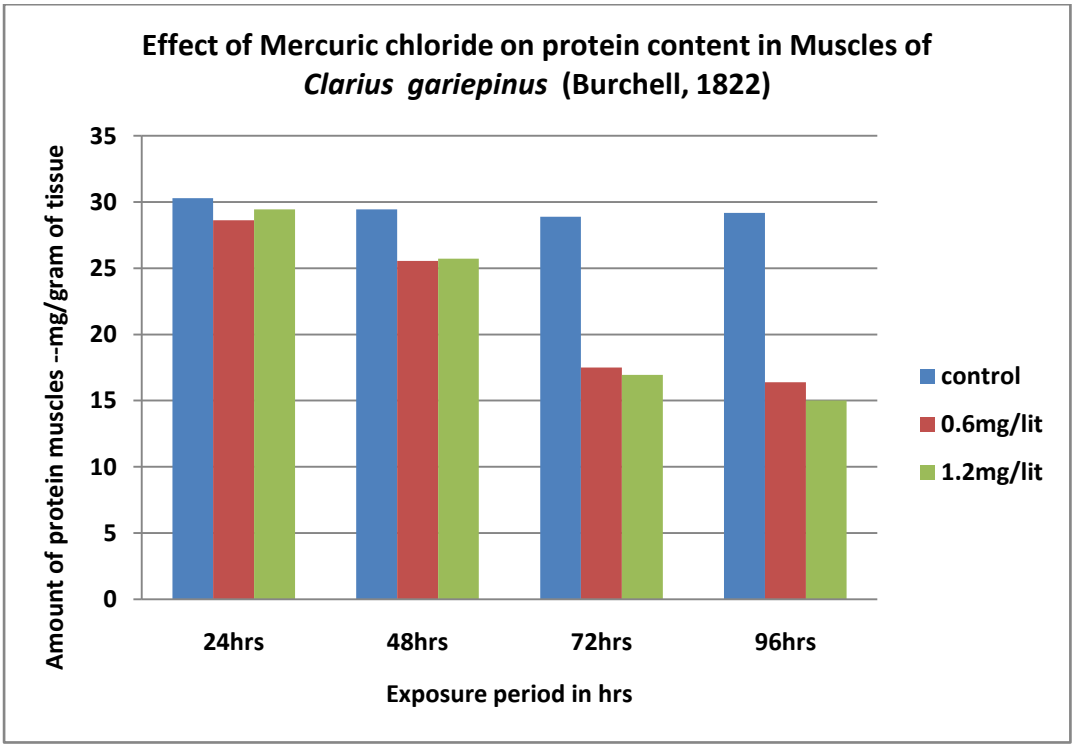


Table-2.

Effect of Mercuric chloride on protein content in Liver of *Clarius gariepinus* (Burchell, 1822)

Concentraions ---in mg/litre	Exposure Time			
	24hrs	48hrs	72hrs	96hrs
Control	29.16655 ± 3.004613	29.44436 ± 3.656553	30.27766 ± 3.367863	28.88837 ± 3.758314
0.6 mg/lit.	29.16655* ± 3.004551	28.05547 * ± 5.091804	26.11101* ± 2.545865	25.27768*** ± 5.67236
1.2 mg/lit.	29.16656 * ± 4.639823	27.49986 * ± 2.204739	22.49991* ± 3.818793	23.22215* ± 3.501334

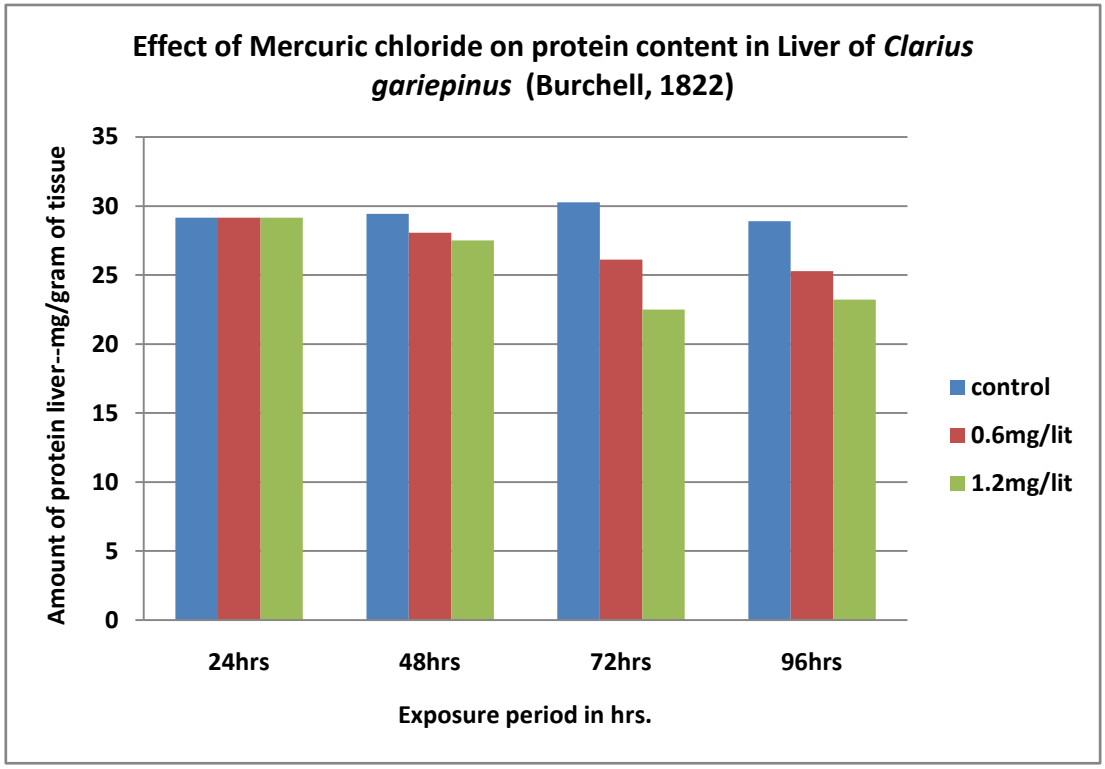
Values expressed as -- --mg /gram of body weight,

± = Standard deviation of three observation.

* = Insignificant, ** = Significant at 5.0%, ***= Significant at 1 %,

ANOVA table was used for calculation.

Graph-2



The protein content in Muscles of *Clarius gariepinus* exposed to mercuric chloride depletion when there is an increase in the concentration of the Mercuric chloride. The protein level shows significant decrease with sublethal concentration of 0.6 mg/L of mercuric chloride at 24, 72 and 96hrs. However, with sub lethal concentration of mercuric chloride i.e.1.2mg/L, protein level significantly decreases from 48hrs onwards. Whereas the amount of protein in liver of *Clarius gariepinus* exposed to mercuric chloride decreases when there is an increase in the concentration of the heavy metals. But it shows significant decrease only with lethal concentration of 0.6 mg/litre of mercuric chloride at 96hrs.

Proteins are highly sensitive to heavy metals and hence indicators of heavy metals poisoning. The protein content in liver is also abundant because of metabolic potential being oriented towards it. Liver is the seat for the synthesis of various proteins besides being the regulating centre of metabolism. The present investigation in the Liver and muscle were justifiable in the wake of mechanical tissue of muscle intended for mobility and does not participate in metabolism. The decreased trend of the protein content as observed in the present study in the fish tissues may be due to metabolic utilization of the keto acids to gluconeogenesis pathway for the synthesis of glucose, or due to the directing of free amino acids for the synthesis of necessary proteins for the maintenance of osmotic and ionic regulation. A dynamic equilibrium exits between proteolysis and synthesis which is mainly responsible for protein turnover and homeostasis in any tissues [13]. The present work agrees with [13] reported that total protein content is decreased and it may be due to breakdown of proteins in the fabrication of some amount of energy for organism. The degree of increase in free amino acids was resulted by the decreased protein level.

Mercuric chloride caused drastic depletion in liver and muscle proteins at all exposure levels. Proteins are highly sensitive to heavy metal poisoning [14]. Depletion in protein level in the exposed fish could be either due to arrested metabolism or owing to its utilisation to build up new cells or enzymes in order to combat the stress [15]. The rapid depletion in total protein content due to active degradation of proteins under stress is reliant on the development of resistance towards the pollutant stress. The decrease of total protein might be attributed to the destruction or necrosis of cells and consequent impairment in protein synthetic machinery [16].

Depletion of liver protein was observed at all concentrations and exposure periods which was highly significant ($p<0.01$) in fish exposed to 1 and 2 ppm of HgCl2 on 14th day, when compared to control. Depletion of protein content in the muscle of fish exposed to mercury chloride for 7 and 14 days in 0.5, 1.0 and 2.0 ppm concentrations were recorded. On 7th and 14^{th} day, highly significant ($p<0.01$) decrease in the muscle protein was observed in fish species exposed to 1 and 2 ppm concentrations of HgCl2, when compared to control [17].

The biochemical content like-carbohydrate, protein and fat content in the body of the fishes decreased with an increase in concentration of household detergents and time of exposure. This decline is due to increase in the metabolic rate and decrease in feeding. As well as an elevated tissue glycolysis, proteolysis and lipolysis, this is contributing to an increase in free glucose, amino acids, fatty acids and glycerol so as to supply the excess demands of energy during the stress conditions and due to tissue damage when there is minimum food intake [18]. The effects of sub lethal concentration of fluoride on the activity of alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) in gills of fresh water fish

Tilapia mossambica was studied. Fluoride acts as an inhibitor of various enzymes like lipases, phosphatases and esterases. The enzyme activities were significantly changed in upon exposure to sublethal concentrations of fluoride due to which protein carbohydrate metabolism was disturbed. In the beginning, carbohydrate concentration was initially increase and later decreases with the time while a significant depletion of total protein and lipids in gills tissue were observed (p 0.001) [19]. The amounts of protein content in muscles and liver is decreased with an increase in concentration of Surf excel and Nirma powder and time of exposure. It indicates protein may be utilized for energy synthesis in tissue and inactivation of enzymes engaged in the protein production and breakdown or increase in proteolysis is affected [20].

Blood serum total protein, serum globulin and serum albumin was analysed every 2hr for 24hrs and again at 48 and 72 hrs. Serum protein and globulin level showed an intial sharp increase from 2 to 20hrs, followed by declines that extend over a period of 72 hrs. Serum albumin showed an initial immediate decline from 2 to 4hrs, followed by an intermittent period of recovery and decline that extend over a period of 72hrs. Both lethal and sub-lethal concentrations of metal salts elicited a similar pattern of response varying only in magnitude

[21]. The toxic effects of mercuric chloride on vital biochemical constituents total glycogen and total protein was also examined. Significant decrease ($p < 0.001$) in glycogen and protein content of fish exposed to 0.077 mgl(-1)was reported [22]. When Zebra fish, *Danio rerio* exposed to mercuric chloride decline in protein level in the gill and muscle was noticed [23].

When an animal is under toxic stress, diversification of energy occurs to accomplish the impending energy demands and hence the protein level is depleted [24]. A significant decrease was reported in muscle and liver protein in *Channa striatus* exposed to mercury, cadmium and lead for a period of 30 days [25]. Depletion of protein content has been observed in the muscle, intestine and brain of *Catla catla* as a result of mercuric chloride toxicity. The depletion of total protein content may be due to the breakdown of protein into free amino acid under the effect of mercuric chloride at the lower exposure period [26]. The rapid depletion in total protein content due to active degradation of proteins under stress is dependent on the development of resistance towards the pollutant stress. The decrease of total protein might be attributed to the destruction or necrosis of cells and consequent impairment in protein synthetic machinery [27].

The protein content was depleted in the muscle, intestine and brain of the (*Catla catla*) exposed to mercury chloride for 24h, 48h, 72h and 96h in 0.1, 0.3 and 0.5m/L sub-lethal concentrations were estimated. Mercuric chloride treated fish shows a gradually decrease in protein level. Depletion of protein was observed at all exposure periods [28]. Measurement of serum biochemical parameters are especially useful to help identify target organs of toxicity as well as the general health status of animals, and is advocated to provide early warning of potentially damaging changes in stressed organisms [29].

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