

COMPARATIVE STUDY OF CHOLESTEROL ALTERATIONS IN A FRESHWATER TELEOST FISH, *AMBLYPHARYNGODON MOLA* EXPOSURE TO HEAVY METALS

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ABSTRACT

Effect of mercuric chloride, arsenic trioxide & cadmium chloride on total cholesterol alteration in liver and gonads of a freshwater teleost fish, *Amblypharyngodon mola* were studied. The fishes were exposed to 0.2291 ppm. mercuric chloride, 1.4557 ppm. Arsenic trioxide and 3.1395 ppm. Cadmium chloride as a chronic treatment. In experimental groups a progressive decrease in total cholesterol content were found with respect to exposure period in liver and gonads after chronic exposure to Mercuric chloride, Arsenic trioxide and Cadmium chloride as compared to control groups.

INTRODUCTION

Ten millions of fish are killed every year by a wide variety of different pollutants from many sources like municipal, agricultural and industrial. From industrial and agricultural operations, these compound find their way in to the natural water resources and affect the aquatic organism (Tilak *et al.*, 2007) Some toxicants contained in the industrial effluents have been reported to be toxic, depending on the dose and exposure duration (Yusuff and Sonibare, 2004), and they can impart serious damage to aquatic life (Vinodhini *et al.*, 2009). The pollutants build up in the food chain are responsible for the adverse effects and finally death of aquatic organisms (Ogundiran *et al.*, 2010). During the stress organisms need sufficient energy which is supplied from reserve material (glycogen, lipid and protein), if the stress is mild then only stored glycogen is used as a source of energy, but when stress is strong then the energy stored in lipids and protein may be used. The heavy metals cause metabolic rearrangement in the living system. Due to their potential toxicity, biochemical changes occur in the organs of animals. (Ram and Sathyanesan, 1984).

Heavy metals have been recognized as strong biological poisons because of their persistent nature and cumulative action. (Joshi *et al.*, 2003; Hoo *et al.*, 2004). Heavy metals have high affinity to sulfhydryl groups and disulfide bonds which

cause damage to secondary structure of proteins and alter the enzymatic activities (Siediecka and Krupa, 2002). The mechanism of action lies in their ability to form strong bonds with bases and phosphates of nucleic acids. They compete with other divalent cations and replace them in their physiological roles (Tabaldi *et al.*, 2007). Ramasamy (1987) has studied the effects of sevin on the lipid content levels of liver and muscle of *Sarotherodon mossambicus* and noted lipids was accumulated. He reported that the lipid levels were much altered when fish was exposed to toxicants (Saravanan *et al.*, 2003). Jha (1991), studied the alteration in the protein and lipid contents of intestine, liver and gonads in the lead exposed freshwater fish, *Channa punctatus*. (Katti and Sathyanesan, 1983) studied the lead nitrate induced changes in lipid and cholesterol level of freshwater fish, *Clarius batrachu*.

Dutta and Haghghi (1986) suggested that heavy metals inhibit cholesterol synthesis in fish. As a result, there is insufficient cholesterol for the maintenance of cell membranes. Cholesterol constitute the vital organic substances, playing an important role in energy metabolism, hence cholesterol from liver and gonads were studied in a freshwater teleost fish, *Amblypharyngodon mola* which is edible fish in North Maharashtra region. Therefore an attempt was made to study the changes in the biochemical composition in different tissues of the fish, when exposed to different heavy metals.

MATERIALS AND METHODS

Live specimens of *Amblypharyngodon mola* were collected from Girna river dams of Chalisgaon, Dist. Jalgaon (M. S.), stored in 60 litre glass aquarium containing dechlorinated water with aeration system. Animals were acclimated to laboratory conditions for 10 days. During the experiment healthy fishes of uniform size and weight were selected for the experiment. Fishes were divided in two groups; one group was maintained as control while the remaining one was separately exposed to the chronic treatment. For chronic treatments the experimental groups of fishes were exposed to 0.2911 ppm Mercuric chloride, 1.4557 ppm. Arsenic trioxide and 3.1395 ppm. Cadmium chloride ($LC_{50/10}$ values of 96 hours) up to 21 days. At the end of 7, 14 and 21 days of treatment, the control and experimental fishes were scarified by decapitation method, liver and gonads were gently separated; used for estimation of their cholesterol in contents. The total cholesterol contents were estimated by using Knobil method. (Knobil *et al.*, 1954). The amount of cholesterol contents was expressed in terms of cholesterol /100 mg. of wet wt. of tissue, each observation was confirmed by taking at least five replicates. The differences in control and experimental animals groups was tested for significance by 't' test (Bailey, 1965) and the percentage decreased or increased over control was calculated for each value.

RESULTS

The depletion in cholesterol contents in all tissues after chronic heavy metals stress were increased as the period of exposure increased. The maximum depletion occurred in the liver followed by gonads. A comparative study shown that the gradual decrease in cholesterol content in various tissues of freshwater teleost fish, *Amblypharyngodon mola* after chronic treatment of heavy metals for various periods of exposure as compared to control. The results are summarised in table-1. The results are also demonstrated that the order of heavy metal effect in cholesterol content in tissues of liver and gonads of *Amblypharyngodon mola*. after chronic exposure, were found that the depletion were more in Mercuric chloride ($HgCl_2$) later on in the Arsenic trioxide (As_2O_3) and then in to the

Cadmium chloride ($CdCl_2$) as compared to control.

DISCUSSION

Amudha P. *et al.* (2002) studied the Dairy effluent induced alterations in the protein, carbohydrate and lipid metabolism of a freshwater teleost fish, *Oreochromis massambicas*, who found that, these concentrations decrease depending on the dose of the effluent. According to Ramana Rao and Ramamurthi (1980), the increase in activity of enzymes lipase is for increasing lypolytic activity to meet the increased demand of energy during stress. Shivaprasad Rao and Ramana Rao (1979), studied that considerable decrease in total lipids in tissue might be due to drastic decrease in glycogen content in the same tissue which is an immediate source of energy during toxic stress condition. After glycogen, lipid content may be used for energy production to overcome toxic stress condition. Some workers support the results in which lipid content decreases. Copuzzo and Lanacaster (1981), reported significant decrease in lipid of post larval lobster when exposed to pollutants. A decrease in total lipid content in tissue reported by Ram and Sathyanesan (1984) on *Channa punctatus* intoxicated with mercuric chloride. The loss of the lipid may be a consequence of inhibition of lipid synthesis and mobilization of stored lipids, Mani and Saxena (1985).

In the present investigation lowering in liver and gonads cholesterol in fresh water teleost fish, *Amblypharyngodon mola*, to an increase in lipid utilization to meet additional energy requirements under stress condition. Lipids may be mobilized to meet the energy requirement of fish either through oxidation or a process of gradual instauration of lipid molecules. In the present work considerable decrease in total lipids in tissue might be due to drastic decrease in glycogen content in the same tissue which is an immediate source of energy during toxic stress condition, significant depletion in cholesterol contents were observed. Similar findings noted by a significant depletion in liver cholesterol content have been reported after Cd treatment of *Garra mullya* for four months (Sinha *et al.*, 2001). Sindhe *et al.* (2002) also reported a decline in cholesterol of both the liver and ovary of *N. notopterus* after exposure to Cd and Hg for two months. Lowering of cholesterol may be attributed to an increase in lipid utilization

Table 1: Impact of heavy metals Mercuric chloride ($HgCl_2$), Arsenic trioxide (As_2O_3) and Cadmium chloride ($CdCl_2$) on Cholesterol content in liver and gonads of *Amblypharyngodon mola* after chronic treatment

Tissue	Treatment	Chronic 7 days	14 days	21 days
Control		23.7114 + 0.004898***	23.6400 + 0.004***	
Liver	Mercuric chloride ($HgCl_2$)	18.7834 + 0.004 898***-21.0210	18.2835 + 0.0074 83***- 22.0210	17.4979 + 0.0063 24***-25.9818
Gonads	Mercuric chloride ($HgCl_2$)	19.7833 + 0.0048 98***-16.8167	19.2119 + 0.0074 83***-18.9761	18.4977 + 0.00743 3***-21.7525
Liver	Arsenic trioxide (As_2O_3)	19.9261 + 0.00 7483***-16.2163	19.1405 + 0.0048 89***-19.2163	18.8548 + 0.0074 83***-20.2419
Gonads	Arsenic trioxide (As_2O_3)	20.2118 + 0.0048 98***-15.0150	19.6405 + 0.006 324***-17.1685	18.8548 + 0.0074 83***-20.2419
Liver	Cadmium chloride ($CdCl_2$)	20.4261 + 0.007 483***-14.1139	19.7833 + 0.004 898***-16.5662	19.5690 + 0.007 483***-17.2208
Gonads	Cadmium chloride ($CdCl_2$)	21.35.45 + 0.00 7483***-10.2103	20.2118 + 0.00 8***-14.7591	19.9976 + 0.0089 44***-15.4077

Values expressed as mg / 100 mg of wet wt. of tissue; (+) or (-) indicate percent variation over control; + indicate S.D. of five observations; Values are significant at $P < 0.001$ ***

for meeting additional energy requirements under stress conditions, as suggested by Srivastava *et al.* (2002). The reduction in hepatic and ovarian cholesterol content may also result in altered vitellogenesis and steroidogenesis, as suggested by Sindhe *et al.* (2002) in *N. notopterus*.

The present investigation concluded that the depletion in the metabolites indicates the fact that whole metabolic pool of the fish gets disturbed / altered under the toxic stress. Further, the change in the biochemical profile indicates their rapid utilization to provide excess energy to cellular biochemical process in order to cope with the stressful condition. The investigatory results of comparative study shown that decrease in cholesterol in liver and gonads were more in mercuric chloride treatment as compared to Arsenic trioxide and decrease in cholesterol in liver and gonads were more in arsenic trioxide as compared to Cadmium chloride as compared to control.

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