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Effect of various plant extracts on ameliorating lipid peroxidation induced by mercury in *Oreochromis mossambicus* (Peters 1852)

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Short communication

Abstract

Lipid peroxidation and the consequent formation of lipofuscin granules have got extremely high importance in heavy metal homeostasis. Lipid peroxidation in fish is found to be an adaptation to internally detoxify and thereby safely assimilate the intruding metals. Though these are of fundamental importance in heavy metal homeostasis, they leave an unfavourable alteration in the physiology of lysosomal lamina. This is evidenced by an increase in the thiobarbituric acid value in fish exposed to mercury. The present study confirmed the efficiency of the aqueous extracts of *Ocimum sanctum, Azadirachta indica, Murraya koenigii* and *Vitex negundo* administered through the feed to mercury-exposed *Oreochromis mossambicus* in alleviating the deleterious effect of the heavy metal.

Keywords*: Lipid peroxidation, lipofuscin granules, thiobarbituric acid value, plant extracts, phytoremediation*

Introduction

Living systems require metal ions for successful performance. Lack of essential metals expresses metabolic defects and mortality in certain cases. However, the ever-increasing influx from different sources has raised the environmental levels of many of the essential and non-essential metals. Once these metals enter the biological system they disturb the biochemistry of different sub-cellular structures.

The heavy metals degrade very slowly and accumulate in the food chain; ultimately hampering aquatic and also terrestrial life. A slight excess of metals in water is extremely toxic to fish. Heavy metal ions precipitate the mucous secretion of the gills in fish (Jezierska and Witeska, 2004). These precipitates occupy the inter-lamellar spaces arresting the movement of gill filaments and blocking their respiratory tract. Mercury has been extensively studied in the field of heavy metal toxicology. Effluents from Chlor-alkali plants and paper and pulp industries bring this hazardous metal into the aquatic environment. Mineral processing and fossil fuel combustion also constitute the anthropogenic sources of mercury. Mercury pollution due to methylmercury is a global problem. Methyl mercury was responsible for the Minamata epidemic in Japan and Sweden. Mercury shows a great affinity for sulfhydryl groups. Mercury poisoning is caused due to the inactivation of several sulfhydryl enzymes thereby damaging cellular structures and leading to catastrophic consequences (Goldwater, 1971).

Among the aquatic fauna, fishes form the most sensitive group. The metal can form complexes with the mucus coating the body surface. At the sub-cellular level, the first detectable onset of toxicity is often associated with lysosomes. From the work of Roch *et al.* (1982) it can be observed that the measurement of the biological responses ideally should be based on the understanding of the intracellular mechanisms. Bayne *et al.* (1988) showed that the toxic effects of chemicals producing diseases in animals occur primarily at the cellular and biochemical levels. Measurement of the extent to which such an alteration has occurred in a given situation would provide a good indication of the significance of toxic effects. It is known that the liver is the main organ where pollutant metabolism occurs and they accumulate to a greater extent in the organ causing its dysfunction due to xenobiotic stress (Eto, 1974). The presence of enlarged lysosomes appeared to be associated with the early phases

of membrane destabilization leading to increased fusion events in the lysosomal vacuolar compartment, as reported by Cajaraville *et al.* (1995). Higher destabilization of the lysosomal membrane as a result of high exposure may result from an enhancement of lipid peroxidation.

Mercury can reduce lipid peroxidation, (Bus and Gibson, 1979) resulting in an augmented attack on the unsaturated fatty acid of the membrane. However, increased heavy metal influx stimulates the lipid peroxidation process and also inhibits the native defence mechanisms involved in the prevention of lipid peroxidation. This alters the normal physiology of the cell and causes the destabilization of membranes. Alterations in the protein content of various tissues of fish *Channa punctatus* exposed to lead were observed by Jha (1991). There is increasing demand for sensitive and specific biological assays to be used in the assessment of the effects of pollutants on the natural population of fish.

The present study aims to find out the ability of the aqueous extracts of *Ocimum sanctum, Azadirachta indica, Murraya koenigii* and *Vitex negundo* administered to mercury-exposed fish through the feed to alleviate the deleterious effect of Mercury. Efforts were made to assess the damage resulting from lipid peroxidation due to mercury on liver cells in terms of thiobarbituric acid value (TBA value).

Material and methods

Live specimens of *O. mossambicus* of average length 10±1 cm and weight 200±20 g were collected from Fort Kochi from the saline waters and were brought to the laboratory immediately. They were acclimatized in large aquarium tanks for one week under defined environmental and nutritive conditions (pH 6.5, temperature 28 °C and dissolved oxygen 3.73 mg/l). The water in the tank was changed daily after the consumption of supplied commercial fish feed. Feeding of fish was suspended 24 hour before the beginning of the experiment and throughout the tenure of the experiment, *i.e.*, 8 days. Fishes were exposed to 1/10th of 96 hour LC50 value of Mercury in a container at a stocking density of five individuals/l of dechlorinated tap water. A control was also run in parallel. The medium of fish was changed every day maintaining the prescribed concentration of mercury after every change. After 96 hours of exposure to the toxicant 2 specimens, each from control and test groups was collected by a net, causing a minimum disturbance. These were immobilized using a blow on the head, the body cavity was cut open and the liver was dissected out. Water adhering to the tissue was blotted off and the weight of the tissue was taken. The extent of lipid peroxidation in terms of the thiobarbituric acid value of the liver tissue was found out following the procedure of Warvedhkar and Saslaw (1959). A weight of 50 mg of tissue was homogenized in distilled water and an equal volume of 50% TCA solution was added. It was centrifuged after half an hour at 1000 rpm for 10 minutes in an ordinary bench-top centrifuge at room temperature. To the supernatant 0.67% TBA was added, shaken well and rapidly cooled. Absorbance was read at 535 nm in a Photochem colourimeter. A reagent blank was also done using distilled water. The results are expressed in n moles of MDA/mg protein. Protein content in the sample was determined by the method of Lowry *et al.* (1951) using BSA as a standard. The remaining fishes exposed to mercury were grouped into five and were subjected to further experimentation as follows. Each group was maintained at a stocking density of 5/l. One group was fed with commercial fish feed for 4 days. The remaining four groups were fed with fish feed prepared by incorporating *Ocimum sanctum* (Thulasi in Malayalam), *Azadirachta indica* (Ariveppu or Veppu in Malayalam), *Murraya koenigii* (Kariveppu in Malayalam) and *Vitex negundo* (Karunochi in Malayalam). A 10 g each of the fresh leaves of these plants were used for feed preparation. The leaves were washed and cleaned and the water content was wiped off. It was then ground well in a mixer grinder into a smooth paste. The fine paste thus obtained is mixed with 100 g rice flour and made into a dough. It is then squeezed through a string hopper maker as noodles or strings. This is dried under the sun. The strings were broken into small pieces of about 0.5 cm and were stored in sealed air-tight packets for further use. All the four plants used for the study are widely used in traditional medicine for various ailments. *Azadirachta indica* is well-known as a herbal pesticide also. *Murraya koenigii* is used for culinary purposes – as a food component as well as for garnishing various curries. After 4 days of feeding, the liver tissue was dissected out and TBA value was determined. The results obtained were tabulated and statistically analyzed for significance using the Student's 't' test.

Results and discussion

The protein content of the tissue obtained was 14.7±0.184, 10.32±0.032, 20.09±0.14, 0.925±0.002, 0.856±0.001, 1.27± 0.0014 and 0.844±0.001 (mg per gm wet weight tissue) for control, mercury exposed, commercial fish feed fed, fed with *O. sanctum*, *A. indica*, *M.koenigii* and *V. negundo* respectively. The thiobarbituric acid value showed a significant ($P<0.001$) increase in the liver of fish exposed to mercury (Fig. 1). Increase in TBA value indirectly pointed to an increase in lipid peroxidation *i.e.*, more and more lipid in the membrane is undergoing peroxidation to disappear from the membrane structure. The increased TBA values obtained in the liver of fish exposed to mercury indicate

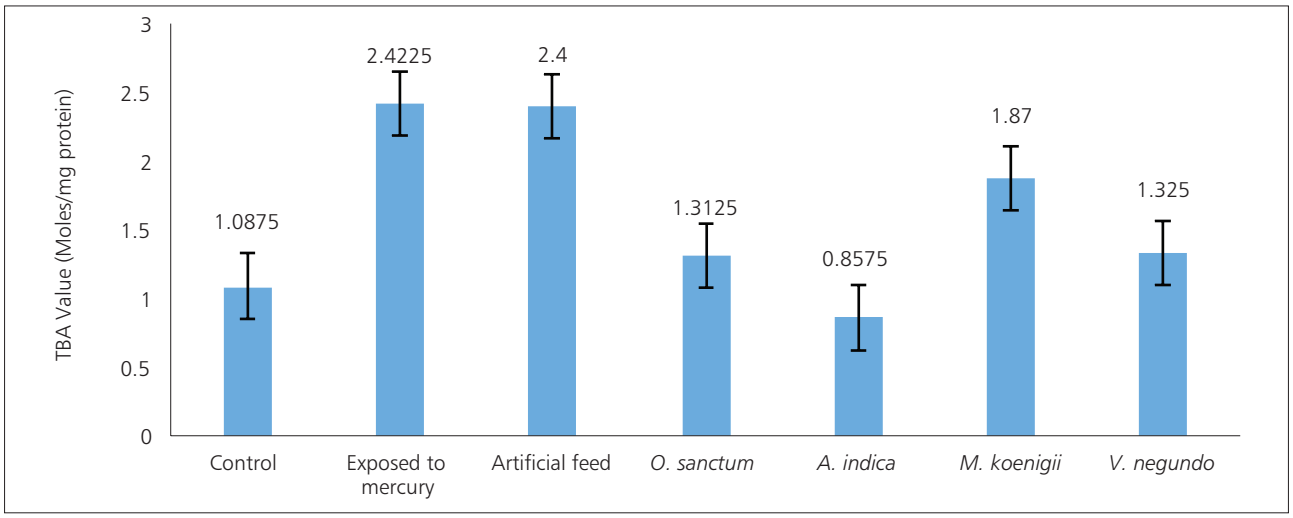

Fig. 1. TBA value in various experimental groups of Oreochromis mossambicus

an increased lipid peroxidation. Mercury entering the cell and hence to the interior of organelles like lysosomes may attack the unsaturated fatty acids of the membrane as an absolute step towards the process of lipid peroxidation. The lipofuscin granules formed by lipid peroxidation bind the toxic heavy metals and make it harmless and unavailable to the cell machinery. But these alternations unfavourably alter the physiology of the membrane causing leakage or liability. Mercury can reduce the activity of these protective machinery, thus enhancing lipid peroxidation (Bus and Gibson, 1979) resulting in an augmented attack on the unsaturated fatty acids of the membrane. Fig. 1 also shows that when the fishes exposed to mercury were fed with commercial fish feed (Control group), the TBA value did not show a significant variation from that of mercury-exposed fishes ($P>0.001$). But when they were fed with feed incorporated with the aqueous extracts of *O. sanctum, A. indica, M. koenigii* and *V. negundo,* the TBA value decreased to 1.3125, 0.8575, 1.87 and 1.325 respectively. All these decreases were significant at a 1% level when compared to the TBA value of mercury-exposed fish.

The TBA value of control fishes was compared with that of fishes fed with feed incorporating aqueous extracts of *O. sanctum, A. indica, M. koenigii* and *V. negundo.* It showed that *O. sanctum, M. koenigii* and *V.negundo* are effective in bringing down the degree of lipid peroxidation in fish exposed to mercury to a considerable extent. But *A. indica* is highly protective and decreases the TBA value even below that of the control fishes.

Conclusion

The works done and the results obtained show that the aqueous extracts of *O. sanctum, A. indica, M. koenigii* and *V. negundo* can ameliorate the ill effects of mercury to varying extents. The results also show that *A. indica* is a very good candidate for curing the damaging effects of mercury and perhaps as a preventive for metal-induced lipid peroxidation.

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