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Original Research Paper

Studies on the Efficiency of Various Plant Extracts in Encountering the Toxic Effect of Mercury on *Oreochromis mossambicus*

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ABSTRACT

Lipid peroxidation and consequent formation of lipofuschin granules have got fundamental importance in heavy metal homeostasis. In other words lipid peroxidation in fish is 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. These alterations are exclusively due to changes in the amount of lipid and protein which make up the lysosomal membrane. This alteration is beyond the permissible limit in fish exposed to mercury. The present paper is aimed to compare the efficiency of commercial fish feed and feeds prepared by incorporating garlic, gooseberry, curry leaves, ocimum and turmeric in remediating the enhanced lipid peroxidation consequent to exposure of fish to mercury. Variations in the level of total protein are also studied.

INTRODUCTION

Water pollution due to heavy metals is a serious menace today. Heavy metals occur naturally in ecosystems with large variations in concentration. In modern times, anthropogenic activities cause excessive introduction of heavy metals to various aquatic ecosystems. Some heavy metals like mercury, cadmium and lead are highly toxic and their accumulation over time in the bodies of animals can cause serious illness. One of the serious problems associated with the persistence of heavy metals is the potential for bioaccumulation and biomagnification, causing heavier exposure for some organisms.

Mercury is the most extensively studied element in the field of heavy metal toxicology. The sources of mercury to the aquatic environment include effluents from chlor-alkali plants and paper and pulp industries. Anthropogenic sources of mercury arise mostly from mineral processing and fossil fuel combustion. It shows little sign of being regulated and a linear relationship between concentrations in seawater and those in fleshes of teleosts has been observed in the field (Gardner 1978). Mercury shows a great affinity for sulphydryl groups and appears to exert toxic effects largely by combining with such groups on protein, thus disrupting enzyme-mediated processes and or damaging cellular structure leading to catastrophic consequences (Goldwater 1971).

Heavy metals produce toxic effects and endanger the life of aquatic fauna. Among these, fishes form the most sensitive group and are obviously the most economically important ones affected. The continuous exposure of both the external and internal organs and almost complete lack of protection of the soft body, through which osmotic influx can occur, make them especially sensitive to the contaminant. The mucus coating of the body surface with which a metal can form complexes aggravates the situation further.

Phytoremediation refers to the use of plants to mitigate environmental problems without the need to excavate the contaminant material and dispose it elsewhere. Phytoremediation results due to the natural ability of certain plants called hyper accumulators to bioaccumulate, degrade or render harmless contaminants in soils, water or air. Many plants such as mustard plants, alpine pennycress and pigweed have proven to be successful at hyper accumulating contaminants at toxic waste sites. Locally available plants such as ocimum, gooseberry, curry leaf, garlic, etc. are also used for phytoremediation.

The present study deals with the use of extracts of some common plants in mitigating the effects of mercury on the fish *Oreochromis mossambicus.*

MATERIALS AND METHODS

Live specimens of *Oreochromis mossambicus* were collected from Matsyafed, Njarakkal, Kochi. They were transported to the laboratory in well aerated tank and acclimated in large aquarium tanks for 1 week under defined environmental and nutritive conditions. The water in the tank was changed daily after consumption of supplied food. Feeding of fish was suspended 24 hours before and throughout the tenure of the experiment.

Five groups of 8 fish were exposed to 1/10th mercury concentration of 96 hours of LC_{50} value i.e., 0.1ppm for 96 hours. A control was also run in parallel. Changes occurring in the thiobarbituric acid value (TBA) of the biological membranes and the content of protein in liver tissue were worked out in control and mercury exposed animals.

After 4 days of exposure to the toxicant, 8 fish each from the control and test groups were collected by a net producing minimum disturbance to the specimen. The fish were immobilized by a blow on the head, the body cavity was cut open and the liver was excised. Lipid peroxidation index or TBA value was determined following the procedure developed by Warvedkar & Sarlaw (1994). The protein content in the liver was measured by the method of Lowry et al. (1951).

Groups of 5 fish from test were subjected to further experimentation. They were kept in containers having de-chlorinated tap water. One group was fed with commercial fish feed for 4 days. The second group of 5 fish was fed with fish feed prepared by incorporating extract of garlic. The experiment was repeated with fish feed prepared from curry leaves, ocimum and turmeric. Results obtained were analysed statistically using Student's *t*-test.

RESULTS AND DISCUSSION

The thiobarbituric acid value show significant increase in the liver of fish exposed to mercury (Table 1). The increase in TBA value indicates that more lipofuschin granules are produced as a result of lysosomal degradation and peroxidation of cellular membrane. A similar result was observed in the gills and digestive glands of *Mytilus galoprovincialis* exposed to copper, cadmium and zinc (Viarengo et al. 1990). In the tissue of Cu exposed mussels, a significant increase in the level of malonaldehyde, indicative of the peroxidative process and an increased accumulation of lipofuschin granules in lysosomes was observed.

In all animals, the cellular membranes are made up of lipoprotein complex. Metals entering into the cell and hence to the interior of the lysosomal membrane may cause oxidative stress, that is produced by the formation of free radicals such as reactive oxygen species (ROS), which include superoxide (O_2), peroxyl, alkoyl, hydroxyl and nitric oxide. ROS are characterised by presence of an unpaired electron in their outer orbit. In addition to these ROS radicals in living organisms are other ROS non-radicals such as the singlet oxygen (O_2), hydrogen peroxide and hypochlorous acid. Small quantities of ROS are formed spontaneously under normal condition as byproducts of redox process such as oxidative phosphorylation in the mitochondria and beta-oxidation of fatty acids. However, the production of ROS is increased when the organism is subjected to irradiation, chemicals or infection (Knapowski et al. 2002). Over production of ROS damages cellular lipids, nucleic acids, proteins and leads to lipid peroxidation (Martin et al. 1996, Finkel & Halbrook 2000). As a result of peroxidation of lipid, lipofuschin granules are formed. These granules bind the metals entering and transform them into an insoluble polymer. These polymers are stored somewhere inside the membrane, thus making it unavailable to the metallic machinery. Usual mechanisms for handling toxic heavy metals include binding to metallothionein and sequestering in lysosomes. The lipofuschin granules formed as a result of lysosomal degradation may be regarded as tertiary lysosomes or residual bodies (George et al. 1982). Viarengo et al. (1990) reports that heavy metals accumulated within the cell stimulate the process of lipid peroxidation.

Mercury is non-essential heavy metal. The significant increase in TBA value for different tissues in fish exposed to mercury may be explained as due to a severe need to keep this non-essential toxic heavy metal away from the metabolic machinery. The lipofuschin granules formed by lipid peroxidation bind the toxic heavy metals and make it harmless and unavailable to the cell machinery. But these alterations unfavourably affect the physiology and membrane causing leakage or liability.

In a cell several defence mechanisms, implicated in the prevention of lipid peroxidation, occur naturally. Mercury is able to reduce the activity of this protective machinery, thus, enhancing lipid peroxidation (Bus & Gibbson 1979) resulting in augmented attack on the unsaturated fatty acids of the membrane.

Table 2 shows that when the fish exposed to mercury was fed with commercial fish feed, and artificial fish feeds, the TBA value significantly decreased. The decrease due to consumption of all the artificial fish feeds was significant at 1% level. This shows the antioxidant activity of garlic, ocimum, gooseberry, turmeric and curry leaf. Fall in the TBA value in fish fed with feeds containing plant extract is supported by the finding of Metwally (2009). Studies of Sharmila Banu et al. (2010) concluded that post arsenic administration of *Ocimum sanctum* has significant role in protecting animals from arsenic-induced oxidative stress and in the depletion of arsenic concentration study by Gaikwad et al. (2010) has also supported the antioxidant activity of plants.

Table 3 shows the effect of mercury on the total protein content in the liver of *Oreochromis mossambicus.* It can be seen that there is a decrease in the protein content from control to test. This indicates that proteins undergo destruction in mercury exposed fish. This is also related to the production of reactive oxygen species ROS, when the organism is

Table 1: The TBA value in the liver of *Oreochromis mossambicus* exposed to mercury.

Sl.No.	Groups	TBA value	*t*-value	Significance
1	Control	0.73 ± 0.024	19.015	1%
2	Test	0.95 ± 0.019		

Values are the mean of 8 different observations ± S.D

Table 2: The TBA value in the liver of fish fed with commercial feed and extracts of garlic, gooseberry, curry leaf, turmeric and ocimum.

Sl.No.	Groups	TBA value	*t*-value	Significance
1	Commercial feed	0.172 ± 0.0022	83.87	1%
2	Garlic	0.444 ± 0.0014	54.69	1%
3	Gooseberry	0.344 ± 0.0014	65.49	1%
4	Curry leaf	0.145 ± 0.0019	86.88	1%
5	Turmeric	0.13 ± 0.014	76.71	1%
6	Ocimum	0.15 ± 0.014	74.83	1%

Values are the mean of 6 different observations ± S.D.

Table 3: The total protein content in the liver of *Oreochromis mossambicus* exposed to mercury.

Sl. No.	Groups	Protein	*t*-value	Significance
1	Control	14.7 ± 0.184	62.05	1%
2	Test	10.32 ± 0.032		

Values are the mean of 8 different observations ± S.D.

Table 4: The total protein content in the liver of fish fed with commercial feed and extracts of garlic, gooseberry, curry leaf, turmeric and ocimum.

Sl. No.	Groups	Protein	*t*-value	Significance
1	Commercial feed	30.7 ± 0.14	363.84	1%
2	Garlic	0.928 ± 0.0014	603.33	1%
3	Gooseberry	0.856 ± 0.0014	607.96	1%
4	Curry leaf	1.27 ± 0.014	549.76	1%
5	Turmeric	0.844 ± 0.0019	608.42	1%
6	Ocimum	0.925 ± 0.0021	603.08	1%

Values are the mean of 6 different observations ± S.D.

subjected to irradiation, chemicals or infection (Knapowski et al. 2002). Overproduction of ROS damages cellular lipids, nucleic acids, proteins and leads to lipid peroxidation (Martin et al. 1996, Finkel & Halbrook 2000). The lipid peroxidation causes overproduction of lipofuschin granules and this leads to the destruction of lipoprotein membrane. Hence, the total protein content is decreased.

Table 4 shows the effect of feeding of different plant extracts on the total protein content in mercury exposed fish. There is a reduction in the TBA value due to the effect of plant extracts, but the protein content is also decreased in these groups. This is mainly caused by an enhanced utilization of protein for growth and hence, the total protein content is decreased. One of the studies conducted in black tiger shrimp larvae support this fact. The high level of enzyme activity obtained with diets containing plant extracts improved the digestion of protein, carbohydrate, fat and cellulose, which might in turn explain the better growth observed with the shrimps fed with plant extract incorporated diet. Similar effect has been reported for fish and shrimp in which digestion was shown to increase considerably in response to probiotics in the diet (Sankar et al. 2011). A study in *Tilapia nilotica* showed that addition of garlic in any form in fish diet can promote growth rate (Metwally 2009).

In fish fed with commercial fish feed, the total protein content is increased. This is due to the presence of some component that is present in food and retards ROS production and also protects the animal from lipid peroxidation. This in turn is responsible for the higher total protein content in this group.

The work done and the results obtained show that the different plant extracts used have varying capacity to irreversibly bind with the heavy metal and make it unavailable to the system. Thus the plant extracts retard or prevents lipid peroxidation and promote growth and survival.

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