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A STUDY ON THE EFFICIENCY OF *PHYLLANTHUS NIRURI* IN AMELIORATING THE DRUG INDUCED OXIDATIVE STRESS IN *Oreochromis mossambicus* AND ITS COMPARISON WITH A HERBAL FORMULA LIV 52

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The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Hepatoprotective and antioxidant potential of *Phyllanthus niruri* and Liv.52, in relieving the paracetamol induced hepatotoxicity in *Oreochromis mossambicus* is compared. Exposure of fish to different concentrations of Paracetamol showed increase in TBA value and a decrease in the total protein content. *Phyllanthus niruri* and Liv.52 supplied through feed probably decreases the reactive oxygen species (ROS) resulting in lower TBA value indicating reduction in lipid peroxidation. Lower protein level in these groups may be due to enhanced utilization of proteins for active metabolism and growth. Fish fed with *Phyllanthus niruri* showed lower TBA value than the paracetamol exposed animals, but the fish fed with commercially produced Liv.52 showed even lower TBA value than those fed with *Phyllanthus niruri*, indicating the greater protective effect of commercially available drug Liv.52 which is a combination of many medicinal plants. The data are statistically analysed using t test.

Keywords: Hepatotoxicity; ROS; *Phyllanthus niruri*; Liv 52.

1. INTRODUCTION

Huge amount of Pharmaceutically Active Compounds (PhACs) are released to the environment due to metabolic excretion, injudicious disposal of industrial waste etc. Paracetamol is one such compound which is extensively used as analgesic and antipyretic drug. However, at high dose it leads to harmful side effects such as toxicity to liver. An effort was made to correlate hepatoprotective and antioxidant potential of *Phyllanthus niruri* and Liv.52, in relieving the paracetamol induced hepatotoxicity in *Oreochromis mossambicus.* This study conducted in the vertebrate

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model can very well be extrapolated to human being also.

The drugs consumed by humans and animals are a cause of real concern nowadays. Most drugs are detected in considerable quantities in drinking water, river waters and sediments, suggesting that pharmaceutical products are of serious concern as widespread pollutants, with possible deleterious effects on human health and the environment. Paracetamol is widely used as analgesic and antipyretic drug, but at high dose it leads to undesirable side effects, such as hepatotoxicity.

Liver diseases have become one of the major causes of distress and mortality all over world. Of these, drug induced liver injury is one of the most common causative factors that causes a major clinical and regulatory challenges. The expression of drug - induced hepatotoxicity are highly variable. It ranges from elevation of liver enzymes which is not accompanied by symptoms, to grave hepatic failure. Cytochrome P450 activates Paracetamol and converts it to toxic metabolite N-acetyl - p-benzoquinoneimine that causes oxidative stress and glutathione (GSH) depletion leading to hepatotoxicity. At higher doses paracetamol causes hepatic necrosis.

The organism selected for the experiment is *Tilapia mossambica*. The industry of rearing tilapia is growing rapidly as they are gaining increasing importance as food fish and are more accepted by consumers in India.

Recently, many authors have outlined the importance of environmental impact assessment programs including methods which measure the biological effects of pollutants on the health condition of organisms [1]. Many studies have been carried out to develop stress indices at different levels of biological organization. These biological responses can be considered as biomarkers of toxicity to central metabolic pathways. Ideally, measurements of biological responses should be based on an understanding of the intracellular mechanisms by which a fish responds to exposure to heavy metals. It would be especially useful if it could be shown that the toxic effects of a metal are related to the perturbations of a particular key biochemical process. Measurement of the extent to which such an alteration has occurred in a given situation would provide a good indication of the significance of the toxic effects [2].

The sublethal cellular pathology induced by xenobiotic, throws light on disturbances of structure and function at the molecular level. In many cases, the earlier detectable changes or primary events are associated with subcellular organelle such as the lysosomes, endoplasmic reticulum and mitochondria. Therefore, explorations at the subcellular level can reveal variations at an earlier stage of response, before integrated cellular damage shifts to the level of organ or whole organism. The structure and or function of organelles and cells can be disturbed by toxic contaminants in many different ways. Slater (1978) has classified these into four main categories, viz, depletion or accumulation of metabolites or co-enzymes, inhibition or stimulation of enzymes and other proteins, activation of a xenobiotic to a more toxic molecular species and disturbances of biological membranes [3]. The cellular responses to pollutant - induced cell injury thus provide rapid and highly sensitive indications of environmental impact. It should also be possible to visualise alterations in the structural and functional organization in individual target cells or groups of cells at an early stage of reaction to cell injury before an integrated cellular response would express at the level of organism and long before the onset of perceptible changes in the population level [4].

From the previous studies it becomes clear that some severe alterations are taking place in the biochemical structure of the lysosomal membrane. It is well documented that the lysosomal membrane which encloses the degradative enzyme are otherwise intact, keeping the organisms "alive". It has been reported that xenobiotics accumulating in the cell, stimulate the process of lipid peroxidation and also impede the native defence mechanism involved in the interruption of lipid peroxidation. This results in the synthesis of lipofuscin granules within the lysosomes and is a principal mechanism in xenobiotic homeostasis. Later on the peroxidation product get converted into an insoluble polymer that sequesters part of the bound xenobiotic. This, then becomes inaccessible to the cell. Thus it is clear that xenobiotic within the cells enhances lipid peroxidation [5].

In spite of massive strides in modern medicine, there are hardly any drugs that invigorate liver function, provide protection to the liver from damage or help regeneration of hepatic cell. Many vital plant extracts are frequently utilized to treat a wide variety of clinical diseases including liver diseases. Therefore, the pursuit for a potent and safe drug for liver disorders continues to be an area of interest. There are however, a number of drugs employed in traditional system of medicine for liver disorders.

The present study was conducted to compare antihepatotoxic and antioxidant potential of *Phyllanthus niruri* and liv.52, in relieving the

paracetamol induced hepatotoxicity in *Oreochromis mossambicus*.

Action of Liv.52, a polyherbal formulation, in regulating the sodium pump in paracetamol induced hepatic injury was investigated by Kala Suhas Kulkarni et al. [6]. Alterations in sodium pump were induced by chronic administration of paracetamol at the dose of 500 and 1000 mg/kg body weight for 28 days. Activity of serum alanine aminotransferase (ALT) and Na -K'-ATPase as well as histology of liver were studied. Chronic administration of paracetamol for 4 weeks to rats led to significant increase in ALT and decrease in liver Na"-K-ATPase activity denoting hepatocellular damage. Results of histological examination was also in agreement with this change with swelling, hydropic degeneration and necrosis of the hepatocytes.

Studies on the protective role of an indigenous drug Liv.52 against a wide variety of toxicants on mammalian organs have been conducted in the past [7–10]. Such studies are lacking in fish. Rathore and Rawat (1989), from their studies have arrived at a conclusion that Liv.52 could revert the Cd-induced histological changes in the gut of mouse [11].

Cadmium poisoning brings about changes in the water quality and fish behaviour, and also decreases the food consumption by fish. Besides, Cadmium also causes structural damage in the fish intestine. It is also demonstrated that Liv.52 provided protection against the degenerative action of Cd and increased the absorptive surface resulting in better food utilization in *M. tengara* [12]. Biochemical studies in mice treated with Liv.52 following Paracetamol treatment showed significant reduction in SGPT levels. Histopathological studies revealed binucleated cells and regenerative activity in 80% livers of mice [13]. Reduction in blood glucose level has been observed by Dwivedi et. al. (1987) in rabbits following carbon tetrachloride administration [14]. The reduction might be due to failure of the damaged hepatic parenchyma to perform their normal mechanism of glucose production. The blood glucose levels returned to normalcy following therapy with Liv.52 in the animals. Liv.52 has hepatoprotective and invigorating actions and also has anabolic effects. The drug has been reported to be efficient in cases of liver diseases in animals by various workers.

Phytoremediation offers a successful, environment friendly and cheap remediation method [15]. The use of recombinant DNA technology to modify plants for metal uptake, transport and sequestration may open up new avenues for improving efficiency of phytoremediation [16]. Agarwal et al, (1986) have reported the hepatoprotective activity of ethanolic extract of roots and leaves of *Phyllanthus niruri* against alcohol induced liver cell injury in partially hepatectomized and non hepatectomized rats [17]. *In-vitro* study of phyllanthin, hypophyllanthin and triacontanol isolated from hexane extract of *Phyllanthus niruri* on CCl_2 and galactosamine induced cytotoxicity in primary cultured rat hepatocytes has shown promising results.

2. MATERIALS AND METHODS

Live specimens of *Oreochromis mossambicus* were collected from Matsyafed, Narakkal. They were transported to the laboratory in well aerated tank and acclimated in large aquarium tanks for 1 week under defined environmental (pH-7.0 and salinity-0 ppt) 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.

Clean containers having a capacity of 50 litres used for the experiment. The stocking density was 5 ltr medium for one fish. The xenobiotic solution and controls were renewed every day to maintain uniform test concentrations. Observations were made after 96 hours of exposure. Four groups of 8 fishes were exposed to paracetamol. Two groups were exposed to 500 mg paracetamol and the other two were exposed to 1g paracetamol per kg of fish. A control was also run in parallel. Changes occurring in the thiobarbituric acid value (TBA) and the content of protein in liver tissue were worked out in control and xenobiotic exposed animals.

After 4 days of exposure to the xenobiotic, 8 fish from the control and 8 from the test were collected by a net producing minimum disturbance to the specimen. The fishes 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 is found out following the procedure developed by Warvedhkar and Saslaw [18]. 50 mg of tissue (liver) was homogenized in distilled water and equal volume of 50% TCA solution was added. It was centrifuged after half an hour for 10 minute in a centrifuge. To the supernatant 0.67% TBA added, shaken well followed by rapid cooling. Absorbance was read at 535 nm in a UV-Visible spectrophotometer. A reagent blank was also done using distilled water. The protein content in the liver is found out by the method of Lowry et al. (1951).

Group of 4 fishes from test were subjected to further experimentation. They were kept in containers having dechlorinated tap water. Of the fishes exposed to 500

mg paracetamol one group was fed with fish feed prepared by incorporating extract of *Phyllanthus niruri* and second group with Liv.52 and the third group with rice flour. The same was repeated with fish exposed to 1g paracetamol per kg of fish. TBA value and protein content after the feeding experiment is also worked as before. Results obtained were analysed statistically using Student's t test.

3. RESULTS

Table 1 shows the TBA value and protein content in the liver of the control and paracetamol exposed *Oreochromis mossambicus*. Table 2 shows the t value and level of significance of the TBA value and protein content in the liver of the control and paracetamol exposed *Oreochromis mossambicus*. Table 3 shows the TBA value and total protein content in the liver of the control as well as paracetamol 500 mg exposed *Oreochromis mossambicus*, which were later subjected to feeding study and their t values and level of significance. Table 4 shows the TBA value and total protein content in the liver of the control as well as paracetamol 1 gm exposed *Oreochromis mossambicus*, which were later subjected to feeding study and their t values and level of significance.

Table 1. Table showing the TBA value and protein content in the liver of *O. mossambicus* exposed to various concentrations of paracetamol

Sl. No.	Group	TBA value (micro moles/gm tissue)	Protein content (mg/g tissue)
1	Control	0.0482±0.0006	68.8±1.6
2	Exposed to 500 mg paracetamol/kg fish	0.4066± 0.0301	54.08± 0.832
3	Exposed to 1 gm paracetamol/kg fish	0.3807±0.004	52.16± 1.298

Table 2. Table showing the t value and level of significance of TBA value and Total protein content in the liver of *O. mossambicus* exposed to various concentrations of paracetamol

Sl. No	Group	TBA value		Protein content	
		t value	significance	t value	significance
1	Between control & Paracetamol 500 mg/kg fish	4.5809	1%	21.1063	1%
2	Between control & Paracetamol 1 gm/kg fish	11.0213	1%	21.8571	1%
3	Between Paracetamol 500 mg/kg fish & Paracetamol 1 gm/kg fish	0.3136	Not Significant	1.0650	Not Significant

Table 3. Table showing the TBA value and protein content in the liver of *O.mossambicus* exposed to 500 mg of paracetamol and later subjected to feeding study

Sl. No.	Group	TBA value (micro moles/gm tissue)	t value	Level of Significance	Protein content (mg/g tissue)	t value	Level of Significance
1	Fed on Rice powder only	0.4088 ±0.006	0.0259	NS	37.6 ± 0.56	31.2316	1%
2	Fed on Rice powder and P. niruri	0.341 ± 0.006	0.8238	NS	21.7± 0.325	67.3100	1%
3	Fed on Rice powder and Liv 52	0.1489 ±0.0045	3.0961	5%	20.68± 0.327	69.3756	1%

Table 4. Table showing the TBA value and protein content in the liver of *O. mossambicus* exposed to 1 g of paracetamol and later subjected to feeding study

Sl. No.	Group	TBA Value (micro moles/gm tissue)	t value	Level of Significance	Protein content (mg/g tissue)	t value	Level of Significance
1	Fed on Rice powder only	0.2554 ±0.0013	0.6285	NS	37.6 ± 0.56	23.8855	1%
2	Fed on Rice powder and P. niruri	0.2082 ± 0.0008	5.6004	1%	26.3± 0.225	46.8568	1%
3	Fed on Rice powder and Liv 52	0.1673 ±0.0019	6.1683	1%	21.26± 1.083	44.7768	1%

4. DISCUSSION

The thiobarbituric acid value shows significant increase in the liver of fish exposed to paracetamol (Table 1). The difference between the TBA value obtained in control and Paracetamol 500 mg exposed fish shows 1% significance. The TBA values of control and Paracetamol 1 g exposed fish also is statistically significant at 1% level. However, there is no significant difference between Paracetamol 500mg and Paracetamol 1 g exposed group (Table 2). The increase in TBA value indicates that more lipofuscin granules are produced as a result of lysosomal degradation and peroxidation of cellular membrane. A similar result was observed in the New Zealand white rabbits exposed to Paracetamol 400 mg/kg body weight [19]. An increase was observed in total leukocyte count, neutrophil count, total lipids, cholesterol, triglycerides, bilirubin, Alanine amino transferase (ALT), Aspartate amino transferase (AST) and BSP% retention. Single overdose of Paracetamol (500mg/kg) produced hepatotoxicity in mice as evidenced by degenerative changes, focal necrosis and sinusoidal dilatation [13]. The SGPT levels were found to be significantly increased by Paracetamol administration in mice. Large areas of necrosis surrounded in focal distribution, lymphocytes and lot of hemosiderin pigment were observed in 67% of animals on histopathological examination. A similar result was observed in the gills and digestive glands of *Mytilus galloprovincialis* [20]. 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 lipofuscin granules in lysosomes was observed.

In all animals, the cellular membranes are made up of lipoprotein complex. A xenobiotic entering into the cell and hence to the interior of the lysosomal membrane may cause oxidative stress by the formation of free radicals such as reactive oxygen species (ROS), which includes superoxide (O_2), peroxyl, alkyl, hydroxyl and nitric oxide. ROS are characterized by presence of an unpaired electron in their outer orbit. Small quantities of ROS are formed spontaneously under normal condition as by-products 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 [21]. Over production of ROS damages cellular lipids, nucleic acids, proteins and leads lipid peroxidation [22-24]. As a result of peroxidation of lipid, lipofuscin granules are formed. These granules bind the xenobiotic entering and transform it into an insoluble polymer. These polymers are stored somewhere inside the membrane, thus making it unavailable to the metabolic machinery. The lipofuschin granules formed as a result of lysosomal degradation may be regarded as tertiary lysosomes or residual bodies [25]. Viarengo et al., (1990) reports that heavy metals accumulated within the cell stimulate the process of lipid peroxidation [20].

Tables 3 and 4 shows the effect of feeding various feeds after exposure to 500 mg and 1 g Paracetamol respectively, on the TBA value and 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. The decrease between control and Paracetamol 500 mg exposed group and that between control and Paracetamol 1 g exposed group is statistically significant at 1% level. However the difference between the protein content of Paracetamol 500 mg and Paracetamol 1 g exposed groups are not statistically significant. This indicates that protein undergo destruction in Paracetamol exposed fishes. This is also related to the production

of reactive oxygen species ROS, when the organism is subjected to irradiation, chemical or infection [21]. Over production of ROS damages cellular lipids, nucleic acids, proteins and leads to lipid peroxidation [22-24]. The lipid peroxidation causes over production of lipofuscin granules and this leads to the destruction of lipoprotein membrane. This may be the reason the decrease in total protein content.

Results show that there is no difference in value between the test and those fed with rice powder. The TBA value of Paracetamol 1g exposed group also shows a little decrease from the value of rice powder fed group and the difference is not statistically significant. This clearly establishes the inert role of rice powder which is used simply as the base in feed preparation. The TBA value decreases in the group fed with extract of *Phyllanthus niruri*. This indicates the efficiency of *Phyllanthus niruri* to decrease in the ill effects of Paracetamol 500 mg and hence a hepatoprotective effect. However it is not statistically significant. The TBA value of fish fed with feed containing Liv 52 is found to decrease from Paracetamol 500 mg exposed group and is statistically significant at 5% level. The fish exposed to paracetamol and then fed with feed containing *Phyllanthus niruri* shows a decrease in TBA value which is statistically significant at 1% level. An equally significant difference is obtained between the fish exposed to paracetamol lg and later fed with feed containing Liv-52. This indicates that the *Phyllanthus niruri* and Liv.52 decrease the ill effects caused by Paracetamol. Fall in the TBA value in fishes fed with the feeds containing *Phyllanthus niruri* is supported by the finding of others [26]. This study was carried out to show the effects of garlic on antioxidant system in Tilapia nilotica, where the malonaldehyde (MDA) formed by lipid peroxidation in plasma decreased significantly.

Sharmila Banu. G (2010) studied about the therapeutic efficiency of oral administration of *Ocimum* in diet after arsenic exposure in rat. The results 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 [26].

Biochemical and histopathological studies revealed that Paracetamol in single oral dose of 500 mg/kg body weight is hepatotoxic in mice. Extract of *Phyllanthus niruri* leaves at a dose of 100 mg/100 g body weight has revealed hepatoprotective activity as is obvious from biochemical & histopathological studies. Moderate hepatoprotective activity was revealed in mice treated with mixture of lignans of *Phyllanthus niruri* at the dose of 50 mg/kg body weight [13]. Decrease in blood glucose level has been observed by Dwivedi et al. (1987) in rabbits following carbon tetrachloride administration [14]. Liv.52 has hepatoprotective and stimulating actions and also has anabolic effects. The drug has been used with success in cases of liver diseases in animals by various workers. Dwivedi et al., (1987) reported good efficiency of Liv.52 in treating experimental liver damage in rabbits.

Table 3 shows the effect of feeding of *Phyllanthus niruri* and Liv.52 on the total protein content in Paracetamol 500 mg exposed fishes. The protein values of Paracetamol 500 mg and rice powder shows decrease in value. Its t value is statistically 1 % significant. The protein value decreases in fish fed with rice powder containing *Phyllanthus niruri* and Liv.52 (1% significant). Table 4 shows the protein value in the liver of *Oreochromis mossambicus* subjected to feeding study after exposing to Paracetamol 1 g. The protein value decreases from fish fed with rice powder to Liv 52. The value between Paracetamol 1 g and *Phyllanthus niruri* exposed group show decrease which is 1% significant. The same is true for Liv 52 treated group also. 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 facts. 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 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 [27]. In-depth hepatoprotective mechanistic study of *Phyllanthus niruri* was conducted by Marwa I. Ezzat, et al. [28]. Another study conducted by Metwally.M.A.A.in *Tilapia nilotica* showed that addition of garlic in any form in fish diet can promote growth rate [25].

5. CONCLUSION

Exposure of fish to different concentrations of Paracetamol showed increase in TBA value. This indicates increased lipid peroxidation probably due to increased production of ROS. Higher lipid peroxidation leads to protein destruction in Paracetamol exposed fishes; hence the total protein content is decreased. *Phyllanthus niruri* and Liv.52 supplied through feed probably decreases the reactive oxygen species (ROS) resulting in lower TBA value indicating reduction in lipid peroxidation. Fish fed with *Phyllanthus niruri* and Liv.52 showed lower protein level which may be due to enhanced

utilization of proteins for active metabolism and growth. Fish fed with *Phyllanthus niruri* showed lower TBA value than the Paracetamol exposed animals, but the fish fed with commercially produced Liv.52 showed even lower TBA value than those fed with *Phyllanthus niruri*, indicating the greater protective effect of commercially available drug Liv.52 which is a combination of many medicinal plants. The general conclusion obtained from the present study is that the *Phyllanthus niruri* play a vital role in lowering lipid peroxidation

COMPETING INTERESTS

Author has declared that no competing interests exist.

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