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EVALUATION OF PROTECTIVE EFFECT OF AQUATIC WEED *HYDRILLA VERTICILLATA* AGAINST LEAD INDUCED ACUTE LIVER TOXICITY IN FISH

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ABSTRACT: The present study is designed to investigate the hepatoprotective effect of *Hydrilla verticillata* on lead-induced hepatic damage in freshwater fish. The fish were treated with a sub-lethal concentration of lead acetate to induce liver damage and fed with supplementary feed containing 20% of hydrilla dry powder. After 21 days of treatment, the liver was removed, and hepatotoxicity and its prevention were analyzed. Liver marker enzymes like alanine transaminase (ALT), aspartate transaminase (AST), acid phosphatase (ACP) were measured and compared with the control. Histopathological study of the liver was done to find out the protective effect of *Hydrilla verticillata*. The lead-induced hepatotoxicity by stimulation of ALT, AST, ACP and represented by degeneration of hepatocytes and formation of cytoplasmic vacuoles. *Hydrilla verticillata* supplementation significantly ($p < 0.05$) reduced the level of hepatic enzymes and showed a protective effect on hepatocytes by normalization of degenerated and fibrotic liver tissue as of control. This study strongly confirms the hepatoprotective effect of *Hydrilla verticillata* against lead-induced acute liver toxicity. *Hydrilla verticillata* could be recommended for liver disorders.

INTRODUCTION: *Hydrilla verticillata* is a submerged aquatic perennial weed which exhibits both monoecious and dioecious form. It is the only species of the genus *Hydrilla*, belonging to the family Hydrocharitaceae. It can grow to the depths of up to 6 meters. It is mostly found in Asia, South America, Australia, and Europe but very abundant in Florida¹. *Hydrilla verticillata* has been recognized as the most invasive weeds².

Hence, efforts are being made to control the economic and ecological impacts of this weed. Surprisingly, it has been noted recently that hydrilla is the most concentrated source of vitamin B₁₂ and also contains large amounts of calcium, which is in the highly absorbable form (100%) with all necessary cofactors. It contains saponin, β - carotene, vitamins, minerals, antioxidants, and detoxifying agents³.

Therapeutically, *Hydrilla verticillata* plant may be used for the following purposes: complete nutrition, improved clearance of waste products of metabolism and detoxification, improved neurological health, improved cardiovascular function and health, effective blood sugar control, slowed ageing, etc.

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The literature survey also reveals that there is a lack of scientific reports on the hepatoprotective effect of *Hydrilla verticillata* in fish. Hence the present study was also designed to evaluate the hepato-protective effect of *Hydrilla verticillata* against the heavy metal - lead, induced liver toxicity in Blue Gourami.

MATERIALS AND METHODS:

Fish Feed Supplementation: *Hydrilla verticillata* dry powder was prepared from the weeds obtained from Kelambakkam Lake, Chennai. The commercial fish feed was grounded in a blender, hydrated with distilled water 2 ml/g of fish feed, mixed with 20% hydrilla dry powder, and extruded through a string hopper machine. The mixture was made into small pellets and air-dried at 70 °C for 48 h in a hot air oven. *Hydrilla verticillata* supplemented fish feed was stored at room temperature ⁴.

Fish Maintenance: The freshwater fish, Blue Gourami (8-10 cm length and 28 ± 0.6 g weight) was used for the toxicity tests. These were collected from ponds of northern districts of Tamil Nadu, India. The fish were acclimatized to laboratory conditions for a week.

The fish were fed with normal feed (Discovery fish feed) and were starved for 24 h prior to the experimentation. Analytical grade lead acetate was used as the metal toxicant. Acute toxicity test for lead was based on the standard method of USEPA, 1995 ⁵. The fish were fed twice a day (2% of the initial body weight per day) with normal feed.

Determination of Median Lethal Concentration (LC₅₀): To evaluate the fish viability and LC₅₀ of lead acetate, the fish were exposed to different concentrations *i.e.* 50, 150, 250, 350, 450, 550, 650, 750, 1000 mg/l of lead acetate.

A stock solution of the test compound lead acetate, and their dilutions were made according to the guidelines given by the Organization for Economic Co-operation and Development ⁶. The mortality rate was determined for 96 h. Then the LC₅₀ was calculated by probit analysis ⁷.

Fish Treatment and Specimen Collection: The 96 h LC₅₀ value of Blue Gourami exposed to lead acetate was found to be 616.46 mg/l. After the

determination of LC₅₀, the fish were exposed to 205.49 mg/l, which is 1/3 value of LC₅₀ for 21 days, to observe the histological changes and study the enzymatic reactions in the liver. Fish were divided into groups as follows:

Group 1: Fed with normal fish feed (Control).

Group 2: Treated with 205.49 mg/l of lead acetate and fed with normal fish feed (Hepatotoxic group).

Group 3: Treated with 205.49 mg/l of lead acetate and fed with fish feed supplemented with 20% of hydrilla dry powder. (Hepatoprotective group). On day 21 of treatment, the fish were sacrificed, and the liver was collected.

Histological Studies: Histological analysis was performed for liver tissues of fish by Hematoxylin and Eosin staining method ⁸.

Liver Marker Enzymes: Liver homogenate was prepared, and the clear supernatant was used for the estimation of enzymes like ALT, AST, ACP using standard kits obtained from Accurex Biomedical Pvt. Ltd, Mumbai.

Statistical Analysis: All the data are expressed as mean ± SEM. Statistical significance of data was analyzed by one-way analysis of variance (ANOVA) followed by Tukey HSD Post Hoc multiple comparisons using the SPSS software package for windows (Version 16). P values < 0.05 are considered as significant.

RESULTS:

Determination of LC₅₀: The fish in the control aquarium were observed to be healthy, normal, and no mortality was recorded. In lead-treated aquarium, no mortality was observed at a lead concentration of 450 and 500 mg/l of lead acetate after 96 h exposure.

However, the fish exposed to the concentrations of 550, 600, 650, 700, and 750 mg/l lead acetate showed 25, 25, 50, 75, and 100 % mortality after 96 h, respectively. It was observed that the percentage and number of survivors decreased with increasing concentration of lead in fish.

The 96 h LC₅₀ for the experimental group of Blue gourami treated with lead was found to be 616.46 mg/l by probit analysis. Lead shows considerable differences in sensitivity amongst fish species.

Effect of *Hydrilla verticillata* on Histological Examinations of Liver: A histological alteration in the liver is a useful biomarker for toxicity studies. The liver consists of a vast inter anastomosing network of hepatocytes arranged in single-cell thick plates separated by vascular sinusoids. In **Fig. 1A** portal tracts are triangular to round structures that contain pre-terminal and terminal portal veins, terminal branches of hepatic artery, and bile ducts embedded in fibrous

connective tissue. In the hepatotoxic group, **Fig. 1B** liver shows a periportal inflammation, feathery degeneration, congestion of blood vessels, degenerative changes in the cytoplasm, and formation of vacuoles. The histology of the fish fed with supplementary feed **Fig. 1C** shows the normal structure of liver in which blood capillaries called sinusoids, forming cord-like structures known as hepatic cell cords, and there is no inflammation observed in the hepatoprotective group.

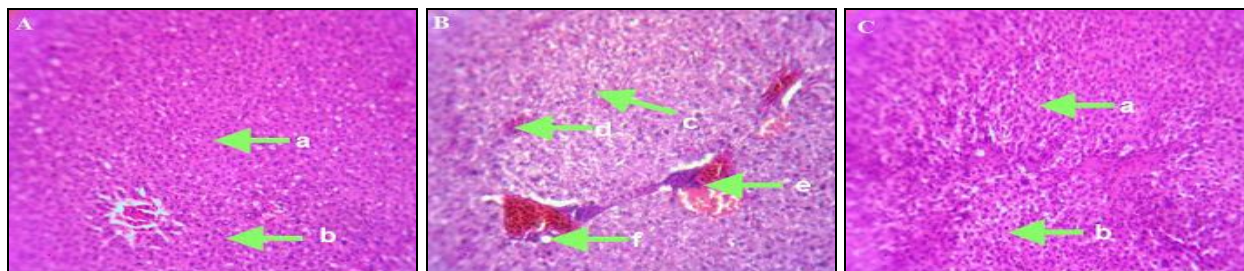


FIG. 1: HISTOLOGICAL ALTERATIONS OF LIVER OF BLUE GOURAMI SHOWING A) NORMAL STRUCTURE OF CONTROL FISH LIVER B) FISH EXPOSED TO LEAD ACETATE AND FED WITH NORMAL FISH FEED C) FISH EXPOSED TO LEAD ACETATE AND FED WITH NORMAL FISH FEED SUPPLEMENTED *HYDRILLA VERTICILLATA*. HEMATOXYLIN / EOSIN STAIN (HE), X 400. A) HEPATIC CELLS, B) SINUSOID, C) HEPATOCYTES FEATHERY DEGENERATION, D) PERIportal INFLAMMATION, E) APPEARANCE OF BLOOD STREAKS, F) VACUOLES IN THE CYTOPLASM

Effect of *Hydrilla verticillata* on Liver Functions: The hepatoprotective effect of *Hydrilla verticillata* on liver marker enzymes such as AST, ALT and ACP against lead-induced liver toxicity was studied. **Fig. 2** clearly shows that 20% of *Hydrilla verticillata* supplementary feed, cures the damages caused in the organs of the fish was observed.

The liver damage induced by lead caused significant ($p < 0.05$) increase in marker enzyme ALT by 421.5%, AST by 631%, ACP by 470.1%. The supplementation of hydrilla significantly ($p < 0.05$) decreased the level of ALT by 47%, AST by 50.2%, and ACP by 65.4% when compared to the hepatotoxic group.

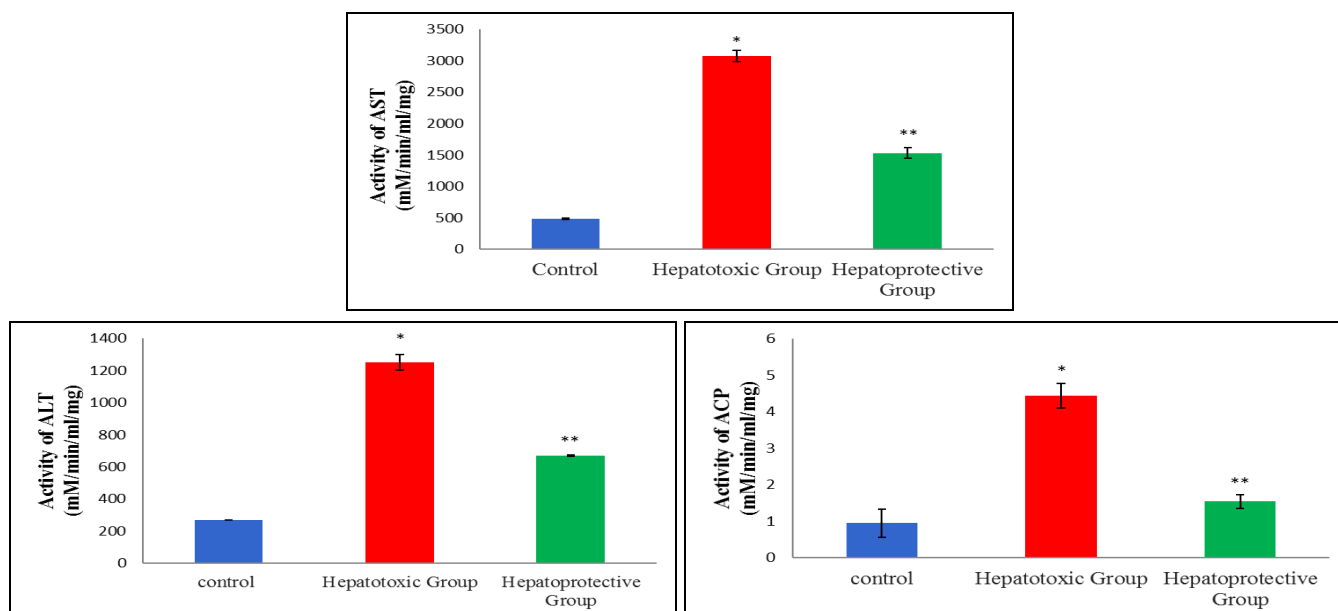


FIG. 2: EFFECT OF SUPPLEMENTARY FEED *HYDRILLA* ON LIVER ALT, AST AND ACP AGAINST LEAD INDUCED TOXICITY IN FISH. VALUES ARE MEAN \pm SD. p Values: * $p < 0.05$ compared with respective control group 1; p values: ** < 0.05 , compared with group 2 (hepatotoxic group)

DISCUSSION: *Hydrilla verticillata* is a rich source of various nutrients, phytochemicals, and antioxidant enzymes like guaiacol peroxidase, ascorbate peroxidase, and catalase⁹. In the present study, it is seen that lead toxicity and liver toxicity were reduced by feeding feed, supplemented with *Hydrilla verticillata*. The supplementary feed contains 20% of hydrilla dry powder. AST and ALT enzymes plays a major role in amino acid, carbohydrate, nitrogen metabolism, and energy generation, particularly gluconeogenesis and TCA cycle. Lead toxicity increased the stress to fish liver tissue and thereby increased the energy demand. This will result in the up-regulation of genes involved in the energy-yielding mechanism and overproduction of enzymes AST and ALT in liver tissues¹⁰.

The elevated levels of AST and ALT indicate that the feeding of amino acids into the TCA cycle occurs in order to cope with the energy crisis during toxicity¹¹. Similarly findings were reported by Offor *et al.*,¹² where high ALT and AST activities were accompanied by high liver microsomal membrane fluidity, free radical generation or destruction of liver tissue. The acid phosphatase is a plasma membrane-derived enzyme that plays a pivotal role in the cytolysis and differentiation process. This may be important in the regulation of the physical properties of membranes or in the absorption of lipids. In the present study, it has been observed that the activity of the ACP enzyme in the tissue of the liver increased significantly as compared to the control group. The elevated level of ACP in different tissues might be due to the increased permeability of plasma membrane or cellular necrosis, and this showing the stress condition of the lead treated fish¹³. Exposed lead could interact with proteins and enzymes of the hepatic tissue interfering with the antioxidant defense mechanism, leading to reactive oxygen species (ROS) generation, which in turn may contribute to the degenerative changes observed in this study.

Due to the presence of antioxidants in the supplementary feed, it may reduce the effects of reactive oxygen species¹⁴. So, the toxicity in the liver may be reduced; it is expressed in the histological changes in the fish fed with supplementary food. The high content of

chlorophyll in *Hydrilla verticillata* supplementary feed increases the activity of phase 2 enzymes involved in the metabolism, which reduces the toxic effect, and hence there is a proportional decrease in the enzyme activity. *Hydrilla verticillata* is loaded with essential minerals and antioxidants that stimulate and support the immune system and rapidly detoxify harmful wastes and toxins¹⁵.

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