Research Article

HEPATOPROTECTIVE EFFECT OF SUPPLEMENTARY FEED *HYDRILLA VERTICILLATA* AGAINST LEAD INDUCED ACUTE LIVER TOXICITY IN FISH

Pandi Prabha S^{1*}, Johanna Rajkumar²

¹Department of Biotechnology, Sri Venkateswara College of Engineering, Pennalur, Sriperumbudur Taluk, Chennai, Tamil Nadu, India

²Department of Biotechnology, Rajalakshmi Engineering College, Thandalam, Chennai, Tamil Nadu, India

*Corresponding Author Email: prabha@svce.ac.in

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ABSTRACT

Objective of the study was to evaluate the hepatoprotective effect of *Hydrilla verticillata* on lead induced hepatic damage in fresh water fish. The fish were treated with 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, liver was removed and hepatotoxicity and its prevention was analysed. Liver marker enzymes like alanine transaminase (ALT), aspartate transaminase (AST), acid phosphatase (ACP), citrate synthase (CS), lactate dehydrogenase (LDH), Hepatic enzymatic and non-enzymatic antioxidants like superoxide dismutase (SOD), glutathione peroxidase (GPx), nitrite, lipid peroxidation (LPO) and lead marker enzymes like aminolevulinic acid dehydratase (ALAD) and cathepsin D were measured and compared with the control. The lead induced hepatotoxicity by stimulation of ALT, ACP, AST, CS, LDH, LPO and oxidative stress by diminution of SOD, GPx, and nitrite. *Hydrilla verticillata* supplementation significantly ($p < 0.001$) reduced the level of hepatic enzymes, LPO and reinstated the defence antioxidant enzymes. The lead toxicity also reduced by the supplementary feed as assessed by decrease in ALAD enzyme and increase in cathepsin D level. This study strongly confirms the hepatoprotective effect of *Hydrilla verticillata* against lead induced acute liver toxicity. *Hydrilla verticillata* could be recommended for liver disorders.

Keywords: Fish, Lead, *Hydrilla verticillata*, Hepatoprotective Effect

INTRODUCTION

Hydrilla verticillata is commonly known as water thyme, Florida elodea, Wasserquirl and Indian starvine which is a native plant of Asia but first introduced to the United States in Florida in 1960. Hydrilla is an aquatic weed and has unique characteristic features to survive in the aquatic habitat. Though this plant can grow in both still and flowing waters, it prefers freshwater lakes, ponds and rivers¹. Therapeutically, *H. verticillata* plant may be used for following purposes: more 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. It contains saponin, β -carotene, vitamins, minerals, antioxidants and detoxifying agents². The literature survey revealed that there is lack of scientific report on the hepatoprotective effect of *Hydrilla verticillata* in fish. Hence the present study was designed to evaluate the hepatoprotective effect of *Hydrilla verticillata* against a heavy metal - lead induced liver toxicity in fish.

MATERIALS AND METHODS

Fish food supplementation

Hydrilla verticillata dry powder was purchased from Lotus Super Foods, USA. The commercial fish food contains 32 % protein, 4 % fat, 4 % fiber, and 11 % ash (Abis fish food, Indian Broiler Group, Chhattisgarh, India). The commercial fish food was grounded in blender, hydrated with distilled water 2 ml/g of

fish food, mixed with 20% hydrilla dry powder and extruded through string hopper machine. The mixture was made into small pellets and air dried at 70 °C for 48 h in hot air oven. *Hydrilla verticillata* supplemented fish food was stored at room temperature³.

Fish maintenance

The fresh water fish, *Labeo rohita* (Rohu) (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 (Abis fish food) and were starved for 24 h prior to the experimentation. After acclimatization, the fish were transferred to aquaria/trough with a capacity of 15 l. The physico-chemical characteristics of water were maintained as recommended by USEPA 1976⁴. Analytical grade lead acetate with purity of 98.89 % (Janaki Scientific Company, India) was used as the metal toxicant. Acute toxicity test for lead was based on the standard method of USEPA, 1995⁵. The fish were fed twice a day (2 % of the initial body weight per day) with normal feed.

Fish treatment and specimen collection

The 96 h LC₅₀ value of *Labeo rohita* exposed to lead acetate was found to be 34.30 ppm using Probit analysis⁶. Then the fish were exposed to 12 ppm lead which is about 1/3 value of LC₅₀ for 21 days. Fish were divided into four groups as follows:

Group 1 served as control, Hepatotoxic group: treated with 12 ppm lead (Group 2), Hepatoprotective group: treated with 12 ppm lead and hydrilla (Group 3), Supplementary feed group: treated only with hydrilla (Group 4). After 21 days, the fish livers were taken and hepatoprotective enzymes such as alanine transaminase (ALT), aspartate transaminase (AST), acid phosphatase (ACP), citrate synthase (CS), lactate dehydrogenase (LDH) and enzymatic antioxidant parameters such as Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and non-enzymatic parameters Lipid peroxidation, Nitrite were studied. The effect on lead toxicity was also evaluated by analysis of tissue delta aminolevulinic acid dehydratase (ALAD) and cathepsin D activity.

Liver marker enzymes

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

Enzymatic and Non-enzymatic Antioxidants

Liver homogenate was prepared and the clear supernatant was used for the estimation of total protein⁷, lipid peroxidases (LPO)⁸, Nitrite⁹ and antioxidant enzymes viz. superoxide dismutase (SOD)¹⁰ and Glutathione peroxidase (GPx)¹¹.

Lead marker Enzymes

The decrease in lead toxicity was evaluated by analysis of tissue delta aminolevulinic acid dehydratase (ALAD) by Berlin and Schaller¹² and cathepsin D activity by using Sapolsky et.al¹³.

Statistical analysis

All the data are expressed as mean $\pm$ SEM. Statistical significance of data was analyzed by one-way analysis of variance (ANNOVA) 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

The hepatoprotective effect of *Hydrilla verticillata* against lead induced liver toxicity was studied. The liver damage induced by lead caused significant ($p < 0.001$) increase in marker enzyme ALT by 223.30 %, AST by 108.67 %, ACP by 139.01 %, CS by 239.21 % and LDH by 154.86 %. Total protein content in the control was 112.25 % higher than the control. The supplementation of hydrilla significantly ($p < 0.001$) decreased the level of ALT by 57.15 %, AST by 34.73 %, ACP by 43.17 %, CS by 62.35 %, LDH by 46.06 % when compared to the hepatotoxic group (Table 1).

Lead intoxication significantly ($p < 0.001$) reduces the antioxidant enzymes SOD by 85.40 %, GPx by 83.44 % and significantly ($p < 0.001$) increases LPO level by 175.06 % and decreases nitrite level by 65.39 %. The results also demonstrated that 441.44 % and 707.03 % increase in SOD and GPx respectively in hepatoprotective group which is supplemented with hydrilla. The % protection in LPO and nitrite by 34.03 % and 72.25 % respectively.

The lead toxicity reduces ($p < 0.001$) the activity of ALAD by 70.43 % and increases cathepsin D level by 214.22 % in lead

intoxicated fish group. When compared to the hepatotoxic group the supplementary feed recovered the ALAD and cathepsin D activity by 111.76 % and 42.86 % respectively.

DISCUSSION

In the present study, it is seen that lead toxicity and liver toxicity were reduced by feeding feed supplemented with *Hydrilla verticillata*. High ALT and AST activities are accompanied by high liver microsomal membrane fluidity, free radical generation and alteration in the liver tissue histogram¹⁴. The rise in the activities of acid phosphatase, citrate synthase due to lead toxicity suggests probable hepatocellular damage in the organism and may be associated with decrease in stability of liver lysosome membrane¹⁵. In most cases of tissue damage, due to toxic compounds, the activity of LDH was reported to be significantly increased¹⁶. Lead induced alteration in redox status as indicated by a decrease in glutathione levels and increase in lipid peroxidation end-product-4-hydroxynonenal levels may be due to damage in RBC membrane and increased LDH in plasma. SOD, the first line of defense against free radicals, catalyzes the dismutation of superoxide anion radical ($O_2^{\cdot-}$) into hydrogen peroxide (H_2O_2) by reduction. The oxidant formed (H_2O_2) is transformed into water and oxygen (O_2) by glutathione peroxidase (GPx). The selenoprotein GPx removes H_2O_2 by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG)¹⁷. When nitrogen monoxide (NO) is formed by NO synthase (NOS), $O_2^{\cdot-}$ will react with NO to form peroxynitrite (ONOO⁻). Both $OH^{\cdot}$ and ONOO⁻ are very reactive and can modify cellular macromolecules and cause toxicity¹⁸. The Pb inhibits the SOD activity. Because SOD is a Zn-Cu containing enzyme, lead exposure induced copper deficiency resulted in decreased erythrocyte SOD activity¹⁹. Followed by SOD, GPx was inhibited. The product of SOD reaction is a substrate for GPx (Fig 1). The expression of GPx is up-regulated by H_2O_2 and other reactive oxygen species (ROS)²⁰. Moreover, Lead is covalently bound to the sulfhydryl groups of glutathione and inhibits the free radical quenching activity of it²¹. The reactive oxygen species ($OH^{\cdot}$) readily attacks the polyunsaturated fatty acids of the fatty acid membrane, initiating a self-propagating chain reaction. The destruction of membrane lipids and the end-products of such lipid peroxidation reactions are especially dangerous for the viability of cells, even tissues²².

δ - aminolevulinic acid dehydratase is a metalloenzyme requiring zinc ions for activity²³. δ -ALAD enzyme is biosensor for lead poisoning²⁴. At the molecular level, lead displaces a zinc ion at the metal binding site, not the active site, producing inhibition through a change in the enzyme's quaternary structure²⁵. So, the δ -ALAD is used to analyze lead pollution in the environment. Cathepsin D is a lysosomal aspartic proteinase. The function of cathepsin D is protein degradation and the molecule has been reported to be involved in vitellogenesis, yolk processing and ovarian follicle growth and maturation. It is also believed to be involved in processing, secretion and activation of enzymes and hormones and in physiological pathways including both extracellular proteolysis and intracellular catabolic proteolysis²⁶. Based on the results, the lead increases the cathepsin D concentration. The actual reason for this is unknown.

The supplementation of dietary vitamins, minerals and phytochemicals are effective against reduction of heavy metals and other toxicity²⁷. The supplementary feed contains hydrilla dry powder. Hydrilla is a rich source of variable nutrients, phytochemicals and antioxidant enzymes like guaiacol peroxidase, ascorbate peroxidase and catalase. It was noted by Pal, hydrilla exhibits detoxification mechanism.

Table 1: Effect of supplementary feed hydrilla on liver SOD (unit/mg of protein/min), GPx (nmole/mg of protein/min), LPO (TBRAS nmole/mg of protein) and Nitrite (nmole/mg of protein) against lead induced toxicity in fish

Groups	SOD	GPx	LPO	Nitrite
Control	6.58 ± 1.16	2.53 ± 0.28	46.44 ± 2.63	26.50 ± 3.35
Hepatotoxic Group	0.96 ± 0.06*	0.42 ± 0.08*	81.30 ± 1.24*	9.17 ± 1.12*
Hepatoprotective Group	5.20 ± 0.38 ^a	3.39 ± 0.04 ^a	53.63 ± 1.39 ^a	15.80 ± 1.67 ^b
Supplementary Feed Group	4.40 ± 0.54	2.89 ± 0.02	42.13 ± 1.47	25.48 ± 3.35

Values are mean ± S.E.M. P values: * P < 0.001 compared with respective control group I; P values: a < 0.001, b < 0.01 compared with group II (Hepatotoxic group)

Table 2: Effect of supplementary feed hydrilla on liver ALT (U/L), AST (U/L), ACP (U/L), LDH (U/L) and Citrate synthase (nm/mg/min) against lead induced toxicity in fish

Fish group	ALT	AST	ACP	Citrate	LDH
Control	121.25 ± 6.85	48.17 ± 6.80	48.74 ± 4.34	0.14 ± 0.02	115.18 ± 2.89
Hepatotoxic Group	392.00 ± 6.02*	100.51 ± 2.01*	116.50 ± 3.97*	0.47 ± 0.02*	293.55 ± 4.50*
Hepatoprotective Group	167.96 ± 8.72 ^a	65.60 ± 4.73 ^a	66.20 ± 2.04 ^a	0.18 ± 0.02 ^a	158.35 ± 0.63 ^a
Supplementary Feed Group	137.16 ± 4.78	51.01 ± 1.46	54.63 ± 3.19	0.15 ± 0.01	139.16 ± 1.02

Values are mean ± S.E.M. P values: * P < 0.001 compared with respective control group I; P values: a < 0.001, compared with group II (Hepatotoxic group)

Table 3: Effect of supplementary feed hydrilla on lead marker enzymes ALAD (µm/mg protein/min) and (Cathepsin D (µm/mg protein/min) against lead induced toxicity in fish

Fish group	Cathepsin D	ALAD
Control	1.16 ± 0.07	0.58 ± 0.04
Hepatotoxic Group	2.49 ± 0.12*	0.17 ± 0.01*
Hepatoprotective Group	1.42 ± 0.11 ^a	0.36 ± 0.02 ^a
Supplementary Feed Group	1.06 ± 0.07	0.55 ± 0.04

Values are mean ± S.E.M. P values: * P < 0.001 compared with respective control group I; P values: a < 0.001, compared with group II (Hepatotoxic group)

Figure 1: Metabolic Pathway in Oxidative Damage¹⁸

Hydrilla contains large amounts of naturally occurring Chlorophyll, which gently removes harmful substances from the body. In hepatoprotective group, the supplementary feed contains antioxidants in it which neutralizes the free radical induced damages in fish liver and reduce the effects of reactive oxygen species^{28,29}. Hydrilla may also react with the protein inhibiting the enzyme involved in the heme synthesis. The phytochemical phytol present in the hydrilla verticillata may help in the protective mechanism. The phytol may interact with ALAD through strong hydrogen bonds. This bonding interaction may reduce the activity of enzyme³⁰. In addition, the protective effect may be due to its ability to induce phase II detoxification pathway via promoting reduced glutathione (GSH) conjugation with toxic metabolites generated from CYP450 pathway³¹ and changes in expression of metabolic proteins CYP1A1, CYP2E, CYP2B and CYP3A.

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