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Antihepatotoxic Activity of *Euphorbia hirta* and by using the combination of *Euphorbia hirta* and *Boerhaavia diffusa* Extracts on Some Experimental Models of Liver Injury in Rats

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ABSTRACT

The antihepatotoxic effect of *Euphorbia hirta* and *Boerhaavia diffusa* extracts were evaluated in experimental models of liver injury in rats induced by CCL_4 or paracetamol. Hydroalcoholic extract (HE) from whole plant were tested. The Hepatic dysfunction was accessed by determining different biochemical parameters in serum and tissues. In serum, the activities of enzymes like Aspartate Aminotransferase (AST), Alanine aminotransferase (ALT), alkaline phosphatase (ALP), alkaline phosphate (ALP), Bilirubin were evaluated. Lipid peroxidation and reduced glutathione were also measured into control and treated rats. *E.hirta* whole plant (HE) showed hepatoprotective activities at doses 125 mg/kg and 250 mg/kg, since serum levels of ALT and AST in rats given the extracts were significantly low ($p<0.05$ and 0.01 respectively) When compare to control CCL_4 or paracetamol-injured rats. Furthered studies were carried on the HE from the whole part of both the plant by using the combination of the extract showed the highest level of antihepatotoxic activity with the hydroalcoholic extract which was effective at doses 75mg/kg and 150 mg/kg, for hepatoprotective activity in CCL_4 and paracetamol-injured rats. In experiments comparing the comprising the HE (125- 250 and 75- 150 mg/kg) to reference antihepatotoxic substance (silymarin) the HE exhibited a 70 and 80% hepatoprotection compared to the 80 and 90% one exhibited by silymarin in CCL_4 or paracetamol -injured rats respectively. This study demonstrated that hydroalcoholic extract *Euphorbia hirta* and *Boerhaavia diffusa* was effective in protecting the liver from toxic hepatitis.

Keywords: Antihepatotoxic activity, *Euphorbia hirta, Boerhaavia diffusa.*

INTRODUCTION

Euphorbia hirta L. is a medicinal, rhizomatous herb distributed in Southern Western Ghats of India and Northern East Coast of Tamil Nadu (Rahuman, and Gopalakrishnan *et al.*, 2007). In East and West Africa extracts of the plant are used in treatment of asthma and respiratory tract inflammations. It is also used for coughs, chronic bronchitis and other pulmonary disorders in Malagasy. The plant is also widely used in Angola against diarrhoea and dysentery, especially amoebic dysentery. In Nigeria extracts or exudates of the plant are used as ear drops and in the treatment of boils, sore and promoting wound healing (Ogueke and Jude *et al.*, 2007). *Boerhaavia diffusa* Linn. (Syn. *B. repens* L.; *B. procumbens* Roxb; family: Nyc- taginaceae, Sanskrit: "Punarnava") is a perennial creeping weed found throughout India. The leaves of *B. diffusa* are reported for their use in the in- digenous system of medicine for the treatment of dyspepsia, jaundice, enlarge- ment of the spleen and abdominal pain (Kirtikar and Basu, 1956). *Boerhaavia diffusa* L. is a wild perennial herb which may be encountered in different terrestrial habitats, ranging from managed grasslands, wastelands, agro-ecosystems to large forest gaps. The species of *Boerhaavia* ('Punarnava') have been in use for medicinal purpose in different parts of India (ICMR 1976). The whole plant, preferably the root, is effectively used to cure several diseases including jaundice (Bajpay 1993; Srivastava & Padhya 1995). In the present communication, an attempt has been made to validate the folklore use of this plant as hepatoprotective against experimentally produced liver injury.

Experimentally, most of the properties reported, but no report so far has been done on the antihepatoxic

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activity *Euphorbia hirta* and *Boerhaavia diffusa* for which is traditionally claimed to heal hepatitis. Carbon tetrachloride and paracetamol are known to cause liver damage (Recknagel, 1983; James *et al*., 2003). When administered to rats, they act by inducing oxidative damages to liver cells which leads to cellular necrosis, resulting in increasing in serum enzymes SGOT and SGPT. These models of hepatotoxicity has been widely used to study the antihepatotoxic activities of exogenous drugs in experimental animal models (Shenoy *et al*., 2001; Bisshayi *et al*., 2002 James *et al*., 2003) In view of conforming the effects on hepatitis, various organic extracts from whole plant of *Euphorbia hirta* and *Boerhaavia diffusa* were prepared and their antihepatotoxic effects evaluated in experimental models of liver injury in rats induced by CCL_4 or paracetamol.

MATERIALS AND METHODS

Animals

Male albino rats (Animal House of Pinnacle Biomedical Research Institute, Bhopal, India.) weighing between 120-150 g were used. They were housed in polypropylene cages under standard conditions (23 ± 2 .C, humidity 60–70%, 12 h light/dark cycles). They were given standard pellet diet and tap water ad libitum.

Preparation of extract: (Solvent – Methanol/Water)

E.hirta was collected locally during the months of September/October from Bhopal, India. Whole plants were washed; air dried under shade and powdered with the help of Grinder at Pinnacle Biomedical Research Institute (PBRI), Bhopal, India. Powdered Whole plant were weighed and packed in soxhlet. Solvent used for soxhletion was mixture of methanol and water in the ratio of 50:50 respectively. Extraction was continued at the temperature of 50°C till clear solvent was observed in siphon tube. Extract was concentrated in water bath at 40°C. Concentrated extract was dried at 40°C in hot air oven. Dried extract was packed in an air tight container and as similar *B.diffusa* was also extracted.

Drug treatment and experimental design:

The following groups of animals were studied:

- Group 1 distilled water control
- Group 2 Received CCl4
- Group 3 Received CCl4 + *E.hirta* at a dose of 125mg/kg body weight
- Group 4 Received CCl4 + *E.hirta* at a dose of 250 mg/kg body weight
- Group 5 Received CCl4 + *E.hirta* and *B.diffusa* in combination at a dose of 75 mg/kg body weight
- Group 6 Received CCl4 + *E.hirta* and *B.diffusa* in combination at a dose of 150 mg/kg body weight

The rats of all groups except group 1 received CCl4 at a dose of 0.1 ml of CCl4 in olive oil (1:1, v/v) per 100 g body weight through an intragastric tube one a week for a period of seven days. The herbal formulation was dissolved in distilled water and given orally through an intragastric tube daily in the morning for seven days.

Collection of serum and tissue samples

At the end of the experimental period, rats were deprived of food overnight and sacrificed. Blood was collected by retro orbital puncture and by the cardiac puncture. It was allowed to clot and then centrifuged at 3000 rpm for 15 min. The serum samples were collected and left standing on ice until required. Tissues (liver) were excised and transferred into ice cold containers for biochemical estimations.

Biochemical evaluation

Activities of serum enzymes such as AST, ALT (Mohun and Cook, 1957) and ALP (Kind and King, 1954) were determined in serum. The concentration of serum bilirubin was also estimated (Malloy and Evelyn, 1937).

Statistical analysis

The statistical significance of difference was tested at 0.05 levels using one-way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Table.1 Effect of *E.hirta* and *B.diffusa* on the activities of enzymes and the concentrations of bilirubin in serum

Treatment dose mg/kg	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	BILIRUBIN (mg/dl)
Control	0.892±1.14	0.545±1.136	0.305±0.007	0.334±0.005
CCL_4-treated	4.417±0.251	3.911±1.124	5.444±0.0132	4.555±1.582
CCL_4+*EH*125	2.842±0.0022	2.514±0.3408	2.087±0.3474	1.607±2.642
CCL_4+*EH*250	1.756±0.6218	1.484±0.00528	0.929±0.06	0.831±0.594
CCL_4+EH + *BD*75	1.256±1.442	1.17±0.979	0.721±0.004	0.541±0.618
CCL_4+*EH*+*BD*150	0.781±0.8134	0.786±1.884	0.565±0.005	0.422±0.053
CCL_4+Silymarin150	0.611±0.8478	0.8133±1.064	0.783±1.148	0.707±0.009

Values are mean ± S.E.M. of six animals in each group

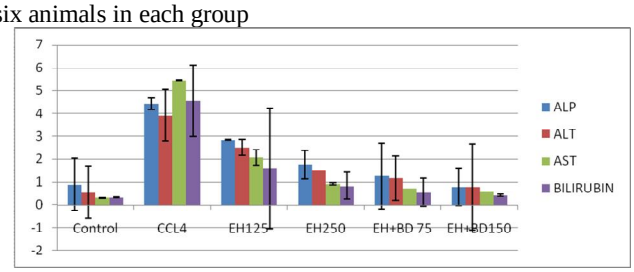
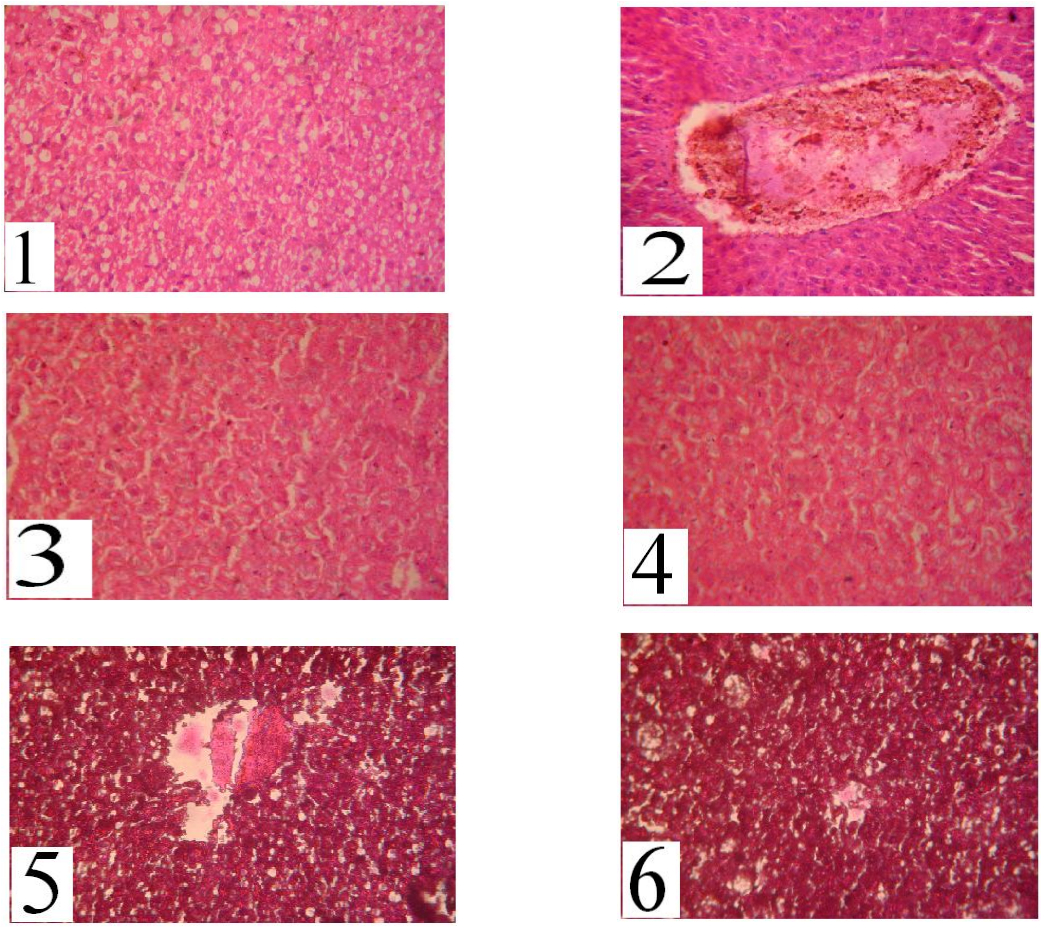


Fig.1 Photomicrographs of liver sections of rat stained with haematoxylin and eosin (x100)



(1) Liver section from normal rat showing normal liver architecture with normal hepatocyte morphology.
(2) Liver section from CCl_4 treated rat showing centrilobular necrosis extending to midzone with neutrophillic collection.
(3) Liver section recovering from CCl_4 induced toxicity in 125 mg/kg treated rat.
(4) Liver section depicting the clear bile canaliculi, normal distribution of kupffer cells and sinusoidal cells in EH 250 mg/kg treated rat.
(5, 6) Liver architecture almost normal in EH and BD in 75 and 150 mg/kg treated rats.

ALP, AST and ALT in serum were increased in CCl4-intoxicated rats. Marked elevations in the concentration of bilirubin, in serum were observed in the hepatotoxin-treated rats. Treatment with *E.hirta* and *B.diffusa* showed a significant protection against CCl_4-induced alterations (Tab.1) in the serum enzyme levels and bilirubin. The degree of protection was observed maximally with the lowest dose of the herbal preparation (i.e., 125 mg/kg body weight and by using combination i.e., 75 mg/kg body weight). The altered biochemical parameters in different tissues were significantly brought towards normalization by co-administration of *E.hirta* and *B.diffusa*. The maximum protection against hepatic damage was achieved with the lowest dose of the drug (i.e., 125 mg/kg body weight and by using combination i.e., 75 mg/kg body weight).

The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiologic mechanisms which have been disturb- ed by a hepatotoxin is the index of its protective effects. The present study reveals that the hydroalcoholic extract of *Euphorbia hirta* and *Boerhauvia diffusa* whole plant possesses such activity against Ccl,-induced hepatotoxicity. The rise in the peripheral levels of certain enzymes, particularly SGOT and SGPT, under the influence of Ccl, has been attributed to the disturbed/damaged structural integrity of the liver (Chenoweth and Hake, 19621. BD seems to preserve the structural integrity of the hepatocyte cell membrane which is evident by a reduction in the Ccl,-induced rise of SGOT and SGPT levels. The observation that treatment with BD before and after CCl_4, poison- ing resulted in a significant decrease in serum bilirubin levels indicates that the drug is effective in the maintenance of normal functional status of the liver.

CCL_4 or paracetamol induced hepatitis are usually used as experimental models in the search for new antihepatotoxic compounds (Fleurentin and Joyeux, 1990). Once introduced in the organism, CCL_4 is converted in the liver into a radical which reacts with

molecular oxygen to form a trichloromethyl peroxyl radical. This compound attacks membrane polyunsaturated fatty acids and causes membrane lipid peroxidation (Recknagel. 1983) which leads to impairment of membrane function. When paracetamol is introduced in excess in the body, this compound is also metabolized in the liver to a reactive metabolite which reacts with enzymes and membrane components of liver cells which result in cellular lesion (Rang *et al*., 1999). In both causes, an increase in the serum of some liver enzymes such as ALT and AST is observed. An extract in acid to be antihepatotoxic if it prevents the increase in the level of these serum enzymes in animals in which hepatitis has been experimentally induced.

In order to efficiently metabolize drugs, during the process of evolution, the liver has developed "drug metabolizing enzymes" which are different from the enzymes of intermediate metabolism (Rao, 1973). Most of these enzymes are largely located in the hepatic microsomes. Biotransformation of a drug or xenobiotic compound following its exposure can alter its distribution and action leading to its detoxification and excretion or enhance its toxicity due to the activation of the compound or due to the biochemical disruption caused by reactive metabolites arising from biotransformation (Athar *et al*., 1997; Plaa, 1991). Biotransformation of xenobiotics usually occurs in two phases. Phase I metabolism (detoxification) involves oxidative, reductive and/or hydrolytic reactions that cleave substrate molecules to produce a more polar moiety. Phase II reactions (synthetic reactions) involve conjugation of certain endogenous molecules to the products of phase I reaction (Remmer, 1970). Cytochrome P450 (Cyt. P450) enzymes are responsible for the metabolic conversion of many drugs to the polar metabolites via Phase I and Phase II reactions to earlier excretion. CCl4-induced hepatotoxicity in rats represents an adequate experimental model of cirrhosis in man and it is used for the screening of hepatoprotective drugs (Al-Shabanah *et al*., 2000; Pérez-Tamayo, 1983; López-Novoa *et al*., 1977). The liver represents the principal site of toxicity, although it induces sublethal proximal tubular injury in the kidney and focal alterations in granular pneumatocytes (Striker *et al.*, 1968).

The biotransformation of CCl4 occurs in the ER and is mediated by Cyt. P450 (Castro *et al*., 1968); the principal isoform implicated as the catalyst being CYP2E 1 (Al-Shabanah *et al*., 2000). Cyt. P450 is inhibited suicidally by the reactive metabolites of CCl4 (Athar *et al*., 1997). CCl3. radical initially formed being relatively unreactive reacts very rapidly with oxygen to yield a highly reactive trichloromethyl peroxy radical (CCl3OO.), which is the probable initiator of lipid peroxidation (Bhat and Madyastha, 2000).

Hepatic cells participate in a variety of metabolic activities and contain a host of enzymes. In tissues, AST and ALT are found in higher concentrations in cytoplasm and AST in particular also exists in mitochondria (Wells, 1988). In liver injury, the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane (Zimmerman and Seef, 1970), thereby causing an increased enzyme level in serum. If injury involves organelles such as mitochondria, soluble enzymes like AST normally located there, will also be similarly released. The elevated activities of AST and ALT in serum are indicative of cellular leakage and loss of the functional integrity of cell membranes in liver (Drotman and Lawhorn, 1978). Administration of CCl4 significantly raises the serum level of enzymes like AST and ALT in rats (Naziroglu *et al*., 1999) as observed in our results. Oral administration of *E.hirta* and *B.diffusa* individually as well as their combination at a dose of 125mg/kg, 250mg/kg, 75mg/kg and 150mg/kg body weight to rats caused a decrease in the activity of the above enzymes, which may be a consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl4. Depletion of elevated bilirubin level together with the suppression of the activity of ALP in the serum of rats treated with *E.hirta* and *B.diffusa*, suggests the possibility of the herbal product being able to stabilize biliary dysfunction of rat liver during chronic injury with CCl4. In conclusion, the hydroalcoholic extract of *E. hirta* and *B. diffusa* whole plant has been shown to be a potent and safe antihepatotoxic drug. Further studies are in progress to isolate and characterize the active principle(s).

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