Research Article

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HEPATOPROTECTIVE ACTIVITY OF *ENHYDRA FLUCTUANS* LOUR. AERIAL PARTS AGAINST CCL₄ INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Enhydra fluctuans Lour. an edible herbaceous vegetable plant and widely used in torpidity of liver and in nervous diseases. The different extracts (Pet-Ether extract, Chloroform extract and Ethanol extract) of *Enhydra fluctuans* aerial parts were studied for its hepatoprotective effect on CCL₄-induced hepatotoxic rats. The extracts were referred as PEEF, CEF and EEF respectively. In the toxicity study, all the extracts did not produce any mortality even at the highest dose (2000mg/kg) so 200mg/kg dose was taken as the tested dose. The extracts were found to decrease significantly CCL₄-induced elevation of SGOT, SGPT, bilirubin and total cholesterol at the dose of 200mg/kg body weight. But it increased HDL-cholesterol level and liver weight with respect to CCL₄ toxic rats. Histopathological profiles of CCL₄-induced hepatotoxic liver revealed extensive centrilobular necrosis extending to other necrotic areas, ballooning degeneration with steatosis. The protective effect of PEEF, CEF and EEF (200 mg/kg, p.o.) were confirmed by histopathological examination of liver section of control, CCL₄-induced and extracts treated groups of rats. Though all the extracts showed remarkable effect. EEF (200mg/kg, p.o.) treated rats exhibited a significant improvement of hepatocellular architecture over CCL₄-induced group as evident from considerable reduction in necrosis and steatosis which is comparable with the standard drug Silymarin (25mg/kg, p.o.).

KEYWORDS: *Enhydra fluctuans* Lour., Hepatoprotective activity, Carbon Tetrachloride, Biochemical studies, Histopathology.

INTRODUCTION

The development of Ayurveda and other traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in the health care¹. Hepatic disorders remain one of the serious health problems. Numerous medicinal plants and their formulations are used for liver disorders in ethno medical practices as well as in traditional Indian medicines². *Enhydra fluctuans* Lour. an edible semi-aquatic herbaceous vegetable plant with serrate leaves grows all over India. The leaves are slightly bitter; cure inflammation, skin disease and good in small pox³. The leaves are also antibilious, used in torpidity of liver and also in nervous diseases⁴. It possesses nutritional value and its methanol extract has been reported to have analgesic activity⁵, anthelmintic and antimicrobial activity⁶. The leaves of *Enhydra fluctuans* have been reported to have hypotensive activity⁷. Chemical constituents like β -carotene⁸, sesquiterpene lactones^{9, 10}, terpenes¹¹ have been reported from this plant. Two new chlorinated melampolides¹² have been isolated and reported recently. Along with this the different plant extracts were reported to contain pharmacologically active phytoconstituents like Flavonoids, Saponins, Steroids, Triterpenoids and Bitter principles¹³ etc. As traditional medicines it is commonly used in India for cooling, carminative, tonic, liver- tonic, leprosy, coughs and especially in hepatopathy¹⁴. In our way to investigate the local medicinal plants for their potential therapeutic uses, present study was aimed to investigate the hepatoprotective activity of petroleum ether, chloroform

and ethanol extract of the aerial parts of *Enhydra fluctuans*.

MATERIALS AND METHODS

Plant Materials

The plant was identified by the taxonomists of the Botanical Survey of India, Govt. of India, Shibpur, Howrah. After authentication fresh aerial parts of the young and matured plants were collected in bulk from the rural belt of Salipur, Odisha, India during early summer, washed, shade dried and then milled in to coarse powder by a mechanical grinder. The powder was passed through sieve number 40 and used for further studies.

Extract Preparation

The powdered plant material was extracted successively with petroleum ether (60⁰-80C), chloroform and ethanol using Soxhlet apparatus. The solvent was removed under reduced pressure which obtained sticky residues. Petroleum ether, chloroform and ethanol extracts were referred as PEEF, CEF and EEF respectively. The dried extracts were stored in desiccators till further study.

Drugs and Chemicals

Silymarin was purchased from Micro labs, Tamilnadu, India, and Carbon tetrachloride (SISCO Research Laboratory, Mumbai). The solvents and other reagents used were of Analytical Grade.

Animals

Studies were carried out using Wistar albino rats (150–180 g) of male sex and Swiss albino mice weighing between 20 and 25 gm. On arrival the animals were placed randomly and allocated to treatment groups in poly acrylic cages (38 × 23 × 10 cm) with paddy husk as

bedding and not more than six animals per cage. Animals were housed at a temperature of $24 \pm 2^{\circ}\text{C}$ and relative humidity of 30-70%. A 12:12 light: dark cycle was followed. All the animals were allowed to free access to water and fed with standard dry pellet diet (Hindustan Lever, Kolkata, India). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (1025/C/07/CPCSEA) and were in accordance with the guidelines of the CPCSEA.

Acute Toxicity Studies

Acute toxicity studies were performed according to OECD-423 guidelines¹⁵. Male Swiss albino mice selected by random sampling technique were employed in this study. The animals were fasted for 4 hr with free access to water only. The PEEF, CEF & EEF extracts were administered orally at a dose of 5 mg/kg initially and mortality if any was observed for 3 days. If mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher (50, 200, 2000 mg/kg) doses of PEEF, CEF and EEF extracts were employed for further toxicity studies.

Experimental Protocols

Healthy albino rats were divided into 6 groups each containing 6 animals. Group-I served as control and received normal saline (2ml/kg body weight, p.o.) once daily. Group-II received 30% CCl_4 in liquid paraffin (1 ml/kg body weight, i.p.). Group-III, IV and V received PEEF, CEF and EEF (200 mg/kg, p.o) respectively and Group-VI received standard drug Silymarin (25 mg/kg, p.o) once in a day and CCl_4 as mentioned above. Treatment duration was 10 days and the dose of CCl_4 was administered after every 72 h¹⁶. Animals were sacrificed 24 h after the last injection. Blood was collected, allowed to clot and serum separated. The liver was dissected out, weighed and used for histopathological studies.

Serum analysis

After 24 h of the last injection, the animals of all groups were anaesthetized and sacrificed. Blood was drawn from heart and serum was separated for the assay of serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT)^{17, 18}, bilirubin (direct and total)¹⁹ and cholesterol (total and HDL)²⁰ using analytical kits from Span Diagnostics Ltd., Surat, India.

Statistical analysis

Statistical significance was determined by One Way Analysis of Variance (ANOVA) followed by Dunnet's t-test to compare group means. The level of significance was $p < 0.001$.

RESULTS

Acute toxicity study

All the doses (5, 50, 200 and 2000mg/kg) of all the three extracts of *Enhydra fluctuans* Lour. employed for acute oral toxicity studies were found to be non-toxic. All the extracts did not produce any mortality even at the highest

dose (2000mg/kg) employed. Therefore the LD_{50} value was more than 2000 mg/kg body weight. In the present study 200mg/kg dose was taken as the tested dose.

Biochemical Estimation

The results of hepatoprotective activity of different extracts of *Enhydra fluctuans* on CCl_4 – treated rats are shown in Table 1. Rats subjected to CCl_4 only developed significant ($p < 0.001$) hepatocellular damage as evident from significant increase in serum activities of GOT, GPT and bilirubin concentration as compared to normal control group which has been used as reliable markers of hepatotoxicity. Oral administration of PEEF, CEF, EEF (200 mg/kg, p.o.) exhibited significant reduction ($p < 0.001$) in CCl_4 -induced increase in levels of SGOT, SGPT and bilirubin (Total and Direct) concentration. Treatment with Silymarin (25 mg/kg, p.o.) also reversed the hepatotoxicity significantly ($p < 0.001$). Table 1 also reveals that total serum cholesterol level of rats treated only with CCl_4 increased significantly ($p < 0.001$) while HDL level decreased significantly ($p < 0.001$) with respect to control group. But PEEF, CEF and EEF were successful in blunting this CCl_4 -induced increase in serum cholesterol level and decrease in HDL level which was comparable with the reference drug Silymarin. The liver weight of rats treated with CCl_4 only decreased significantly ($p < 0.001$) which was blunted by the extracts and Silymarin. Histopathological profiles of CCl_4 -induced hepatotoxic liver revealed extensive centrilobular necrosis extending to other necrotic areas, ballooning degeneration with steatosis (Photograph 2). The protective effect of PEEF, CEF and EEF (200 mg/kg, p.o.) were confirmed by histopathological examination of liver section of control, CCl_4 -induced and extracts treated groups of rats. Though all the extracts showed remarkable effect, EEF (200mg/kg, p.o.) treated rats exhibited a significant improvement of hepatocellular architecture over CCl_4 -induced group as evident from considerable reduction in necrosis and steatosis (Photograph 5). Liver section of rats treated with Silymarin (25mg/kg, p.o.) showed significant signs of amelioration of CCl_4 -induced liver injury which was evident from normal liver architecture and absence of necrosis and steatosis (Photograph 6). The study showed that EEF and Silymarin showed significant protective effect against CCl_4 -induced liver injury which was evident from their histopathological examination.

DISCUSSION

Out of all the hepatotoxin, CCl_4 is the most commonly used hepatotoxin in the experimental study of liver disease²¹. It is well documented that carbon tetrachloride is biotransformed under the action of cytochrome P_{450} in the microsomal compartment of liver to trichloromethyl radical which readily reacts with molecular oxygen to form trichloromethylperoxy radical²². Both the radicals can bind covalently to the macromolecules and induce peroxidative degradation of the membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the pathological changes such as elevated levels of serum marker enzymes such as SGOT, SGPT and bilirubin²³.

Table 1: Effect of PEEF, CEF, EEF (200mg/kg, p.o.) on SGOT, SGPT, bilirubin, cholesterol and liver weight on CCl₄ induced hepatotoxicity in rats

Group	Treatment	SGOT(U/ml)	SGPT(U/ml)	Bilirubin (mg/dl)		Cholesterol (mg/dl)		Liver weight(g)
				Total	Direct	Total	HDL	
I	Control	54.16 0.91	46.00 0.81	0.57 0.02	0.080.1	101.83 1.58	29.98 0.19	10.90 0.50
II	CCl ₄ treated	138.003.58 ^{##}	126.66 2.98 ^{##}	6.69 0.48 ^{##}	1.380.05 ^{##}	199.165.12 ^{##}	2.11 0.11 ^{##}	7.42 0.29 ^{##}
III	CCl ₄ + PEEF	82.33 3.37 ^{**}	78.83 3.72 ^{**}	1.42 0.06 [*]	0.69 0.03 ^{**}	131.33 2.87 ^{**}	19.44 0.13 ^{**}	10.10 0.31 ^{**}
IV	CCl ₄ + CEF	72.00 3.10 ^{**}	76.83 3.51 ^{**}	0.88 0.08 ^{**}	0.58 0.02 ^{**}	124.83 1.90 ^{**}	20.45 0.12 ^{**}	10.17 0.34 ^{**}
V	CCl ₄ + EEF	68.16 2.65 ^{**}	70.33 2.17 ^{**}	0.61 0.02 ^{**}	0.23 0.02 ^{**}	114.66 2.89 ^{**}	23.43 1.80 ^{**}	10.20 0.36 ^{**}
VI	CCl ₄ + Silymarin (25 mg/kg)	63.83 2.52 ^{**}	56.33 2.41 ^{**}	0.58 0.02 ^{**}	0.20 0.03 ^{**}	112.83 1.97 ^{**}	24.16 0.13 ^{**}	10.36 0.30 ^{**}

All values are Mean±SEM, n=6 rats in each group^{##} $p < 0.01$ as compared with Group I and ^{*} $p < 0.05$ and ^{**} $p < 0.01$ as compared with Group-II by Dunnetts multiple comparison

Photograph 1: Section of liver tissue of normal rat showing normal liver architecture with central vein and portal triads (H & E, 400x)

Photograph 4: Section of liver tissue of rat treated with Chloroform (CEF) (200mg/kg, p.o.) showing abnormal liver architecture with no necrosis, but less steatosis and mild sinusoidal congestion (H&E400x)

Photograph 2: Section of liver tissue of rat induced with CCl₄ showing extensive centrilobular necrosis, extending to other necrotic areas, ballooning degeneration along with fatty degeneration or steatosis (H & E, 200x)

Photograph 5: Section of liver tissue of Ethanol extract (EEF) treated (200 mg/kg, p.o.) rat showing almost no necrosis, diffuse steatosis and mild increase in inflammatory cells in portal tract (H & E, 400x)

Photograph 3: Section of liver tissue of rat treated with Pet-Ether (PEEF) extract (200mg/kg, p.o.) Showing abnormal liver architecture with steatosis and huge sinusoidal congestion (H & E, 400x)

Photograph 6: Section of liver tissue of rat treated with Silymarin (25 mg/kg, p.o.) showing almost normal liver architecture with no necrosis, no steatosis and mild sinusoidal congestion (H&E,400x)

The assessment of liver function can be made by estimating the activities of these serum enzymes. During liver damage there may be an increase in these enzyme levels in serum with the extent of liver damage. The elevated or altered levels of these enzymes in the CCl₄ – treated experimental animals in the present investigation corresponded to the extensive liver damage induced by the hepatotoxin.

The hepatoprotective activity of PEEF, CEF and EEF were monitored by estimating serum transaminases and bilirubin which give a good idea about the functional state of the liver²⁴. Necrosis or membrane damage releases the enzymes into circulation and therefore it can be measured in serum. The increase in the level of serum bilirubin reflected the depth of jaundice and increase of serum transaminases was a clear indication of cellular leakage and loss of functional integrity of cell membrane²⁵. Our results demonstrate that PEEF, CEF and EEF caused significant inhibition of SGOT, SGPT and bilirubin levels when compared to CCl₄-induced hepatotoxic rats. Effective control of levels of serum transaminases and bilirubin level points towards an early improvement in secretory mechanism of hepatic cells.

Results of the present studies, suggest that as compare to PEEF and CEF the EEF extract has an ability to protect the liver from CCl₄-induced liver damage. The protective activity of the extract may be attributed to the membrane stabilizing agents present in the plant which may avert enzyme leakages in tissues in response to CCl₄ poisoning²⁶. Certainly further studies need to be carried out with other hepatotoxic compounds to prove the hepatoprotective efficacy along this, studies are needed to isolate and characterize the compounds responsible for the above effect.

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