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The possible hepatoprotective activity of *Eclipta alba* whole plant extract against CCl_4

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ABSTRACT

The hepatoprotective activity of different extracts of whole plant *Eclipta alba* was investigated against CCl_4 induced hepatic damage in male albino rat. The extract (500 mg/kg) administered for 7 days were compared with the standard silymarin (Silybon -70, 10 mg/kg,b.w.). The petroleum ether, chloroform and methanol extract have shown significant hepatoprotective activity by reducing the elevated levels of serum enzymes such as serum glutamate oxaloacetate transaminase(SGOT), serum glutamate pyruvate transaminase(SGPT), alkaline phosphatase (ALP) and serum bilirubin, and elevation in total protein (TP) . These biochemical observations were also supplemented by histopathological examination of the liver sections, where the methanol extract was found to be potent among the three extracts.

KEYWORDS

Anti-hepatotoxic;
Carbon tetrachloride;
Eclipta alba.

1. INTRODUCTION

Eclipta alba Hassk., syn. *Eclipta prostrata* L._Asteraceae.is a small branched herb with white flower, which is found in moist places throughout India and tropical and sub-tropical regions of the world. Commonly, it isknown as 'black bhringraj' when in fruit and as 'white bhringraj' when in flower[1]. *Eclipta alba* is traditionally used for jaundice in India[2,3]. As a reputed herbal medicine, it is ingredient of number of antihepatotoxic phytopharmaceutical formulations in the Ayurvedic and Unani system of medicine. This drug is also used for removing obstruction of the tubules of kidney and ureters[4], and is claimed to be useful in osteoarthritis of the knee[5]. It also cures vitiligo (leucoderma)[6] and insomnia[7]. The drug also showed antiviral activity in mice experimentally infected with Semliki forest encephalitis virus[8]. Phytochemical investigations revealed the presence of coumastanes, polypeptides, polyacetylenes,thiophene-derivatives, steroids, triterpenes, flavonoids and nicotine[9-13].

The present study is focused to evaluate the hepatoprotective potentials of the *Eclipta alba* against CCl_4-induced liver injury in rats.

2. MATERIAL AND METHODS

2.1 Plant collection

Eclipta alba were collected from the market of Khari Baoli, Chandi Chauk, Old Delhi. The plant was authenticated by Dr. M.P. Sharma, Reader and taxonomist, Department of Botany, Hamdard University, New Delhi. A voucher specimen of plant was kept in herbarium of Hamdard University, New Delhi.

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2.2 Preparation of extract

Coarsely powder dry *Eclipta alba*(5kg) were extracted to exhaustion with petroleum ether (60-80°C), chloroform and methanol using a soxhlet apparatus successively. The extracts thus obtained were dried under reduce pressure yielding 11.4%, 27.8%, 34.6% with reference to dry starting material respectively.

2.3 Experimental animals

Male albino rats of wistar strain (150-200g) were maintained under controlled condition of light (12/24h) and temperature (23±1°C). Food pellets (Hindustan lever Ltd. Mumbai, India) and tab water were provided ad *libitum*. For experimental purposes animals were kept fasting but were allowed free access to water.

2.4 CCl_4- induced hepatotoxicity

The animals were divided into six groups of six animals each. Group I served as normal control received only normal saline. Group II served as CCl_4 control and received CCl_4: liquid paraffin (1:1, 1.5 ml/kg,b.w., p.o.) on first day. Group III served as standard control and received single dose of CCl_4: liquid paraffin (1:1, 1.5 ml/kg,b.w., p.o.) on first day and thereafter received treatment with standard drug silymarin (Silyb on-70)(10mg/kg, b.w., p.o.) for 7 days. Groups IV-VI received single dose of CCl_4: liquid paraffin (1:1, 1.5 ml/kg,b.w., p.o.) on first day and thereafter treated with petroleum,chloroform and methanolic extract of *Eclipta alba* (500 mg/kg, b.w., p.o.),[14] respectively, for 7 days.

2.5 Assessment of liver functions

Rats of all groups were anaesthetized with 1.2 g/kg b.w, of a 25% w/v aqueous solution of urethane (Loba-Chemie, Bombay), given on 8th day. The blood collected by puncturing the orbital plexus was allowed to coagulate at ambient temperature for 30 min. and the rats were sacrificed by decapitation. Serum was separated by centrifugation at 3500 rpm for 10 min. The livers of all animals were removed and processed for histological investigations. In serum, alanine aminotransferase (ALT), aspartate aminotransferase (AST)[15], alkaline phosphatase (ALP)[16], total protein (TP)[17] and total bilirubin[18] were measured.

2.6 Histological observation

Liver slices fixed for 48 h in 10% formosaline were processed for paraffin embedding following the standard microtechnique[19]. Sections (5 /~m) of livers stained with haematoxylin and eosin were evaluated for histopathological changes under a light microscope.

2.7 Statistical analysis

The data were expressed as mean ± S.E.M. (n = 6). Results were analyzed statistically by one-way ANOVA followed by Dunnett's test. The difference was considered significant if $p < 0.05$.

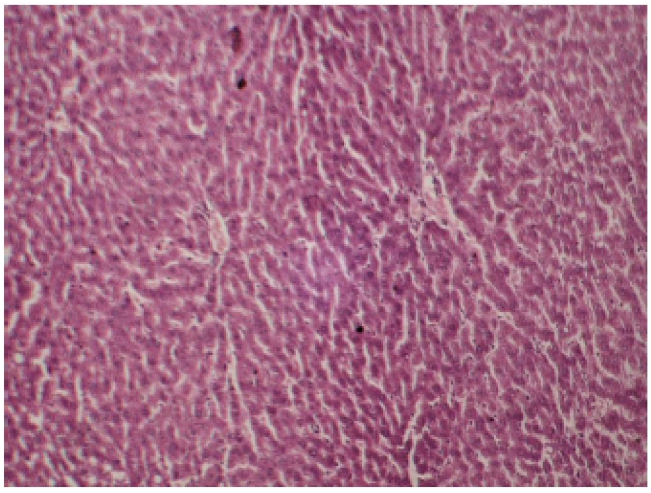

Figure 1a : Histology of the liver of control rat showing normal hepatic cells architecture

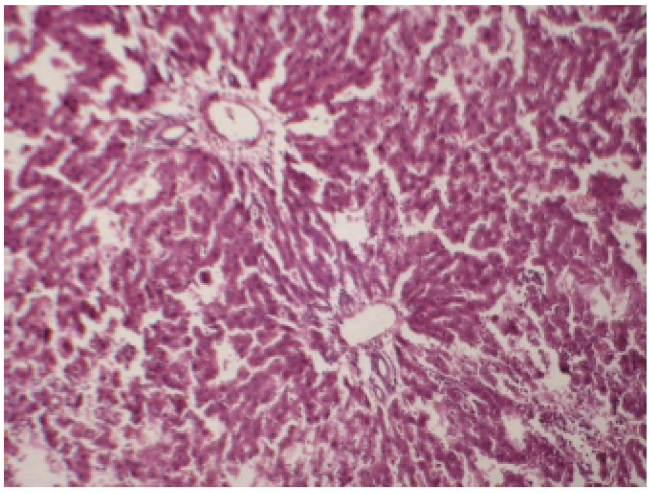

Figure 1b : Histology of the liver of carbon tetrachloride-treated rats showing necrosis with the obliteration of architecture in hepatic cells

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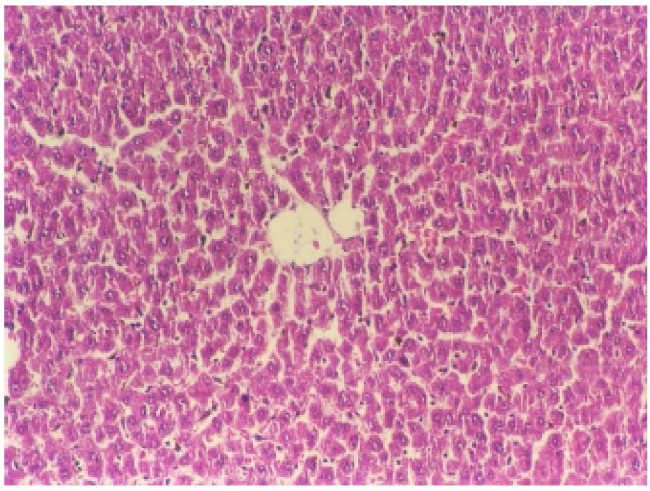

Figure 1c : Histology of the liver treated with silymarin showing recovery of the hepatic cells

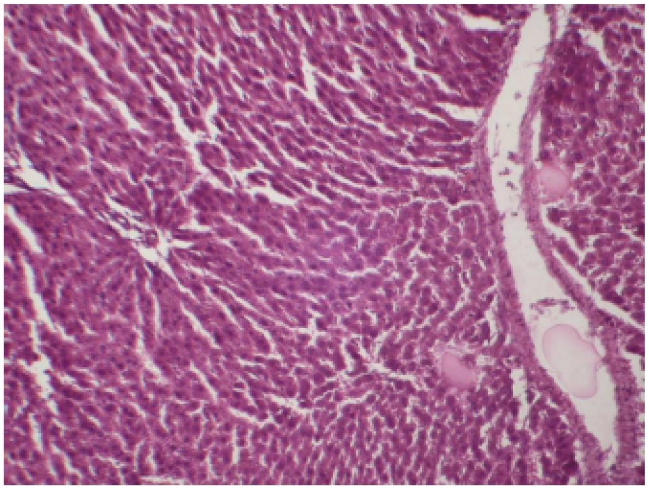

Figure 1d : Histology of the liver treated with petroleum extract of *Eclipta alba* showing recovery of the hepatic cells

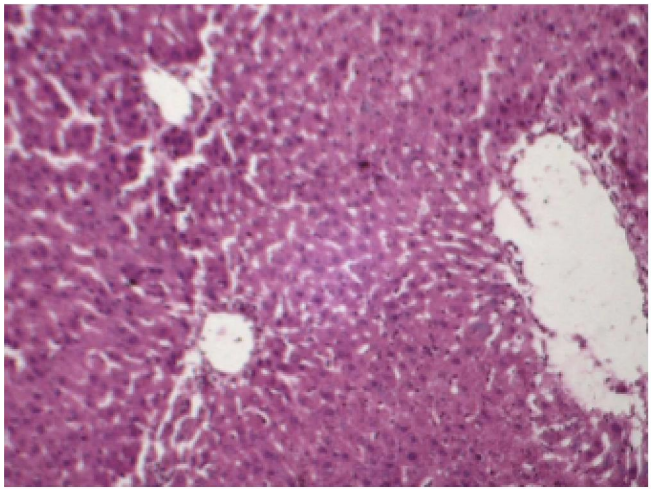

Figure 1e : Histology of the liver treated with chloroform extract of *Feronia limonia* showing recovery of the hepatic cells

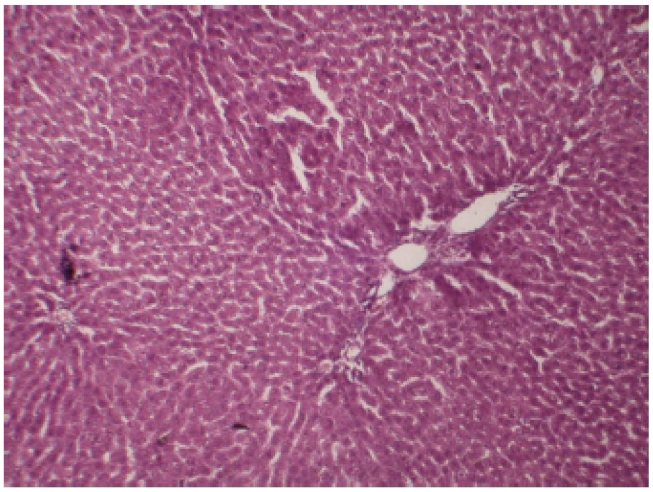

Figure 1f : Histology of the liver treated with methanolic extract of *Eclipta alba* showing recovery of the hepatic cells

TABLE 1 : Effect of various fractions of *Eclipta alba* on serum enzymatic activity in CCl_4 induced liver damage in rats

Group	Treatment	Dose	SOGT units/ml	SGPT units/ml	ALP units/ml	Total Protein (g/dl)	Total Bilirubin (g/dl)
I	Normal control	---------	59.49±2.405**	48.22±2.87**	47.71±1.62**	7.47±0.32**	2.12 ± 0.20**
II	Toxic control	1.5 ml/kg(p.o.)	175.54±3.807	134.94±5.106	82.06±2.078	4.84±0.25	3.91 ± 0.07
III	Silymarin (Standard drug)	10 mg/kg(p.o.)	95.148±3.277**	76.007±3.648	51.45±1.44**	6.98±0.18**	2.23±0.18**
IV	Petroleum ether fraction	500mg/kg(p.o.)	133.33±2.27**	107.75±2.95**	69.14±2.43*	5.44±.11*	2.37 ± 0.12**
V	Chloroform fraction	500mg/kg(p.o.)	115.46±2.13**	94.67±4.60**	64.622±1.66^{ns}	5.97±.15**	2.31 ± 0.15**
VI	Methanol fraction	500mg/kg(p.o.)	99.72±2.34**	86.26±2.44**	57.97±1.36**	6.212±0.18**	2.26 ± 0.23*

****P<0.01; *P<0.05 vs CCl_4 ; P>0.05 ns.**

Value are mean ± S.E. of five animals. One way analysis and Dunnett's test.

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3. RESULT

The effect of extract of *Eclipta alba* on serum transaminases, alkaline phosphatase, bilirubin and total protein levels in CCl_4 intoxicated rats are summarized in TABLE 1. There was a significant in-crease in serum SGOT, SGPT, ALP and bilrubin levels. The total protein level was significantly decreased in CCl_4 intoxicated rats. The extracts of *Eclipta alba* at the dose 500 mg/kg orally significantly decreased the elevated serum marker enzymes and reversed the altered total protein to almost normal.

Histological observation of liver tissue of the normal animal showed hepatic cells with well-preserved cyto-plasm, nucleus, nucleolus, and central vein. In CCl_4 treated group, histological observation showed fatty degeneration, damage of parenchymal cells, steatosis and hydropic degeneration of liver tissue. Prominent damage of central lobular region appeared in the liver. The extracts of *E.alba* restored the histopathological abnormality induced by CCl_4.

4. DISCUSSION

Carbon tetrachloride is one of the most commonly used hepatotoxins used in the experimental study of liver diseases[20]. It was found that administration of CCl_4 produced liver cirrhosis in rats. It is well documented that carbon tetrachloride is biotransformed under the action of cytochrome p 450 - 2e1 (CYP2e11) in the microsomal compartment of liver to trichloromethyl (*CCl3) and peroxytrichloromethyl (Cl3COO*) free radical[21-23]. These free radicals 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 formation of lipid peroxides followed by pathological changes such as depression of protein synthesis, elevated levels of serum marker enzyme such as SGPT,SGOT and ALP.

The elevated levels of serum enzymes are indicative of cellular leakages and loss of functional integrity of cell membrane in liver. Serum ALP and total bilirubin levels on the other hand are related to the function of hepatic cells[24]. Extracts have significantly decreased the serum SGOT,SGPT towards normal level. These indicate that extracts preserved the structural integrity of the hepatocellular membrane and liver cell architecture damage caused by CCl_4, which is confirmed by histopathalogical studies.

CCl_4 intoxication also produced significant rise in serum bilirubin thereby indicating hepatic damage[25]. It is well known that necrotizing agents like CCl_4 produce sufficient injury to hepatic parenchyma to cause elevation in bilirubin content in plasma[26,27]. These effects induced by CCl_4, were confirmed by our results. The extracts at the dose of 500 mg/kg orally for seven days significantly restored the altered ALP and total bilirubin levels. CCl_4 intoxification significantly lowered total protein levels and at 500 mg/kg extract orally significantly increased protein levels and also preserve the structural integrity of the hepatocellular membrane and liver cell architecture damaged by CCl_4, which was confirmed by histopathalogical studies.

In summary, the results of this study demonstrate that methanol extract of *Eclipta alba* has a potent hepatoprotective action on $CC1_4$ induced hepatic damage in rats. These results show that the hepatoprotective effects of extracts of *Eclipta alba* may be due to its ability to block the bioactivation of $CC1_4$ by inhibiting P450-2e1 and improving the structural integrity of the hepatocyte..

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