Hepatoprotective Activity of *Aeschynomene Aspera* Linn

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Summary

The hepatoprotective activity of benzene and alcoholic extracts of root of *Aeschynomene aspera* was investigated in rats for carbon tetrachloride induced hepatotoxicity. LD50 values for both extracts determined. The extracts did not produce any mortality even at 5000 mg/kg while LD 50 of benzene and alcoholic extracts was found to be 100 mg/kg and 200 mg/kg. Hepatotoxicity was induced in rats by intraperitoneal injection of carbon tetrachloride (1 ml /kg /day diluted with olive oil (1:1) for 3 days). Benzene and alcoholic extracts of roots were administered to the experimental rats (100 and 200 mg/kg/d p.o for 3d). The Hepatoprotective effect of these extracts was evaluated by liver function biochemical parameters (total bilirubin, serum protein, alanine aminotrnasaminase, aspartate aminotransaminase and alkaline phosphatase activities) and histopathological studies of liver. In benzene and alcoholic extracts –treated animals, the toxicity effect of carbon tetrachloride was controlled significantly by restoration of the levels of serum bilirubin and enzymes as compared to the normal and standard drug silymarin - treated groups. Histology of liver sections of the animals treated with the extracts showed the presence of normal hepatic cords, absence of necrosis and fatty infiltration which further evidence the hepatoprotective activity.

Key Words: *Aeschynomene aspera,* Benzene extract, Alcoholic extract

Introduction

The liver is the main organ regulating homeostasis in the body. It is the most involved with all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction [Ward & Daly]. The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals.

In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate [Pang et al 1992 and Ross et al 1996]. Presently only a few hepatoprotective drugs and that too from natural sources (there is not a single effective allopathic medicine) are available for the treatment of liver disorders.

Aeschynomene aspera is a shrub that grows up to 150 centimeter found in the eastern and the southern regions of India. The species of Aeschynomene has been traditionally curative agents in colic, jaundice and poisoing [Nadkarni 2002]

Although *Aeschynomene aspera* comes under the medicinal list, no work has been made yet on this plant. But *Aeschynomene indica* was found to contain flavonoid reynoutrin, and amino acid potassium aeschynomate [Fekndnfullas 1996]. There is paucity of scientific evidence regarding its usage in liver disorders. Hence, the present study was aimed to investigate the hepatoprotective activity of root extracts of *Aeschymomene aspera* in carbon tetrachloride induced hepatoxic model in rats.

Materials and Methods

Drugs and Chemicals

Silymarin was obtained from microlabs, Bangalore. The kits for all biochemical estimations were purchased from Transasia Biomedical Ltd., Daman India. The solvents and other chemicals used were of analytical grade.

Plant materials and extracts

The roots of *Aeschynomene aspera* were collected during Sep-November of 2006 from the field of Nellianthalpatty, Tamilnadu; were authenticated by a taxonomist Dr. Stephen, American College, Madurai and a voucher specimen [C-H26] deposited in the department of pharmacology, Ultra college of Pharmacy, Madurai,Tamil Nadu,India. The roots were shade dried at room temperature and ground thoroughly in grinding mill to get a coarse powder. The powdered material was successively extracted with benzene and alcohol by hot continuous percolation method in soxhlet apparatus. The extracts were dried at 50^{o}C in a water bath. The percentage yield of benzene extract of *Aeschynomene aspera* (BEAA) and alcohol extract of *Aeschynomene aspera* (AEAA) were 3.4 % w/w and 10.18 %w/w respectively.

Phytochemical Screening

A preliminary phytochemical screening of BEAA and AEAA was carried out as described by Khandelwal KR et al [2000].

Animals

Wistar albino rats (120-150gm) of either sex were procured from Ultra college of Pharmacy animal house, Madurai and were acclimatized for 10days under standard housing conditions maintained at a room temperature of 24 ± 1^{o}C; relative humidity of 45.55 % with 12:12 hour light/dark cycle. The animals had free access to rat feed [Amuruth rat feed, Bangalore] and water *ad libitum*. The animals were habituated to laboratory conditions for 48 hours prior to the experimental protocol to minimize any non specific stress. The institutional Animal Ethics committee of Ultra college of Pharmacy Madurai, India, approved the experimental protocol in accordance with the guidelines provided by committee for the purpose of control and supervision of experiments on animals (CPCSCA) with registration no. U /08/04/U/ CPCSCA.

LD_{50} determination

Acute oral toxicity (AOT) of BEAA and AEAA were determined using nulliparous, non pregnant female mice. The animals were fasted for 3 hour prior to the experiment and were administered with single dose of extracts dissolved in 10% W/V Tween 80 and observed for mortality for upto 48 hrs (short term toxicity). Based on the short term toxicity, the dose of the next animal was determined as per OECD guideline 425.

Hepatoprotective activity

BEAA and AEAA at doses of 100 mg/kg and 200 mg/kg and Silymarin at a dose of 100 mg/kg were administered orally to rats of the respective groups three times at 12 hrs intervals. Control animals received vehicle, carbon tetrachloride diluted with Olive Oil (1:1) was administered in a dose of 1 ml/kg body weight for 3days to all animal groups except for control [Pulok K.Mukherjee 2005] and animals of the untreated group received only CCl_4 to assist assessing the severity of toxicity produced by carbon tetrachloride administration. After 72 hrs carbon tetrachloride treatment, blood was collected from all groups of rats by puncturing the retro-orbital plexus. Serum was separated by centrifugation at 2500 rpm at 37^{o}C for 15 minutes and analyzed for various biochemical parameters. Histopathology of liver was carried out by a modified method of Luna et al [1999]. In brief, the autopsied livers were washed in normal saline and fixed in 10 % formalin for 2 days followed with bovine solution for 6 hrs. Then the livers were paraffin embedded and 5µ thickness minoton section made. The sections were processed in alcohol xylene series and stained with haemotoxylin and eosin. The slides were studied under a light microscope for any histological damage / protection.

Statistical analysis

The data are expressed as mean ± SEM statistical differences between mean were determined by one way ANOVA followed by Tukey Kramer's post hoc test. Values of $P<0.05$ were considered as significant.

Table1 Effect of *Aeschynomene aspera* Linn root extracts on CCl_4 induced hepatotoxicity in rats.

Groups (n)	Dose mg/kg	Total Bilirubin (mg %)	Total Protein (gm %)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	Vehicle	1.53 ± 0.053	8.38 ± 0.12	36.16 ± 3.954	315.83 ± 24.88	74.5 ± 8.03
CCl_4	3 ml	2.16 ± 0.11	6.98 ± 0.05	542.16 ± 43.04	738.666 ± 0.02	169.83 ± 7.58
Silymarin	100	0.93 ± 0.07**	7.71 ± 0.06***	260.33 ± 23.22*	319.5 ± 6.307***	147.16 ± 16.91*
Benzene extract	100	1.25 ± 0.051**	7.1± 0.05***	111.66 ± 4.16**	155.5 ± 10.88***	102.33 ± 3.34*
Alcoholic extract	200	1.2 ± 0.057*	7.1 ± 0.07***	160.66 ± 3.676*	196.83 ± 23.26***	189.16 ± 5.05*

Values are expressed as Mean ± SEM, n = 6 rats in each group.

* $P < 0.01$, ** $P < 0.001$, *** $P < 0.05$ compared to standard group.

Results

Preliminary phytochemical studies revealed the presence of steroid, carbohydrates, and flavonoid in BEAA while steroid, alkaloid and flavonoid were noticed in AEAA. The BEAA was found to be nontoxic up to a dose of 1000 mg/kg and the LD_{50} of AEAA was found to be 2000 mg/kg, Administration of carbon tetrachloride to rats caused significant liver damage, as evidenced by the altered serum biochemical parameters. Pretreatment of rats with BEAA and AEAA exhibited marked protection against carbon tetrachloride induced hepatotoxicity, which is shown in Table 1. Hepatocytes of the normal control group showed a normal lobular architecture of the liver (Fig 1). In CCl_4 treated group the liver showed micro vascular fatty changes and the hepatocytes were surrounded by large number of fat droplets (Fig 2). Silymarin – BEAA and AEAA pretreated groups showed minimal fatty changes and their lobular architecture was normal, indicating the hepatoprotective effect of these extracts (Fig 3-5). However, BEAA showed less micro vascular fatty changes than AEAA. The hepatoprotective activity of the extracts were in the order of silymarin >BEAA > AEAA.

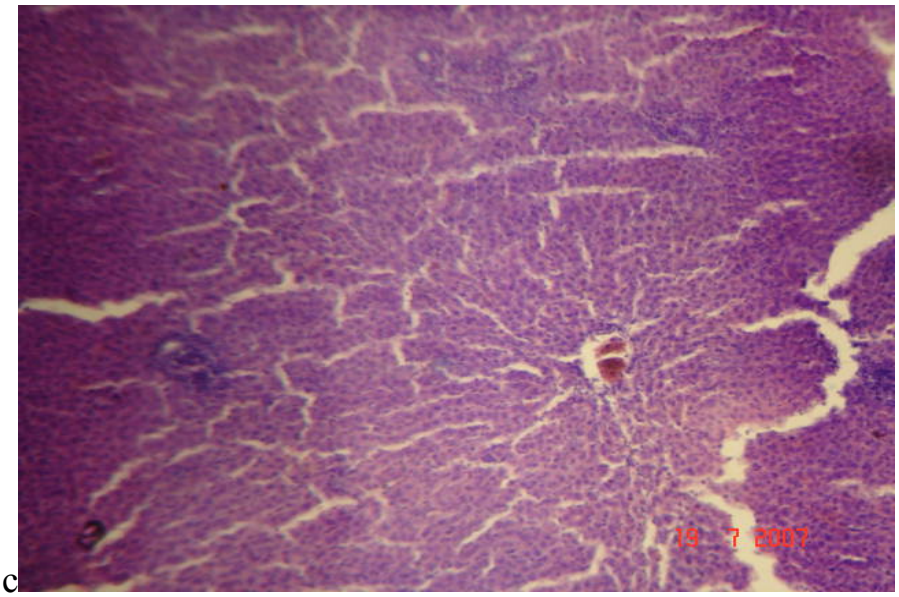
c 

Fig 1 Section of the liver tissue of control rats showing normal histology, normal liver lobules with central vein and normal portal triad

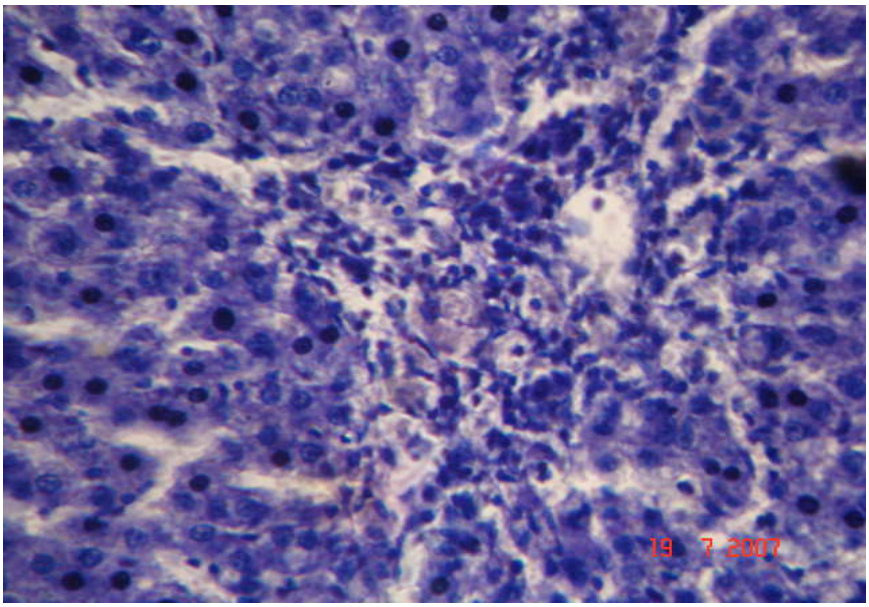


Fig 2 Section of the liver tissue of CCl_4 treated rats showing inflammatory cells in the portal triad and fatty vacuole with necrosis

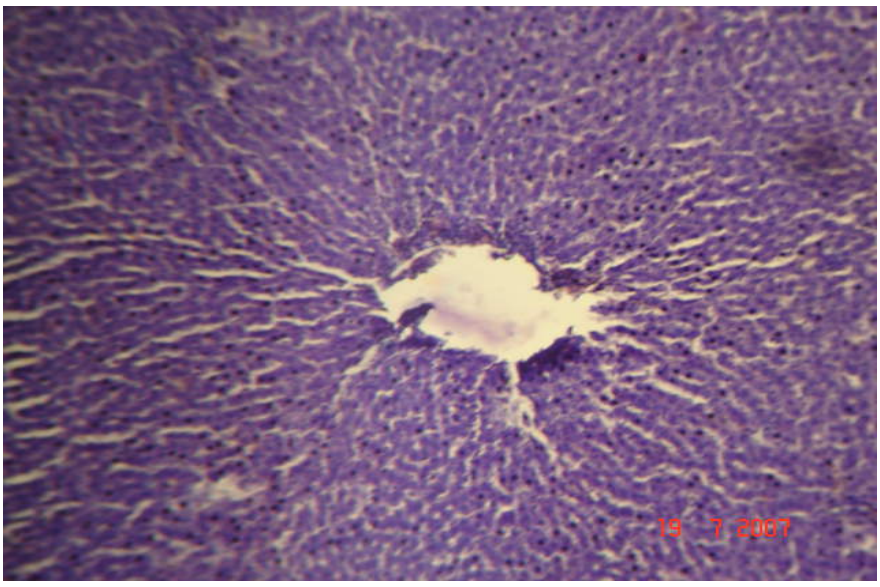


Fig 3 Section of the liver tissue of Silymarin treated rats showing normal hypatocytes around central vein, normal lobule and portal triad

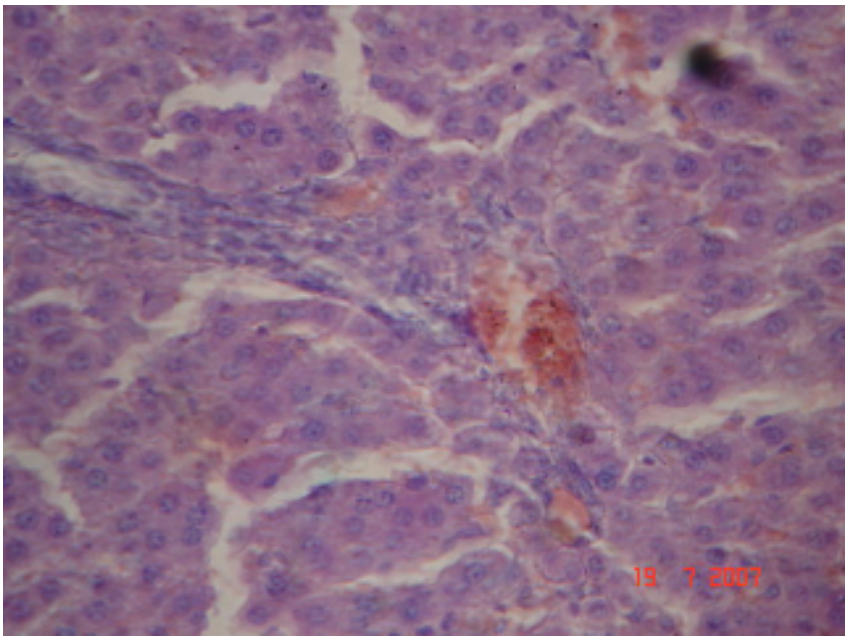


Fig 4 Section of liver tissue of benzene extract treated rats showing normal hypatocytes around central vein with little fatty change and absence of necrosis

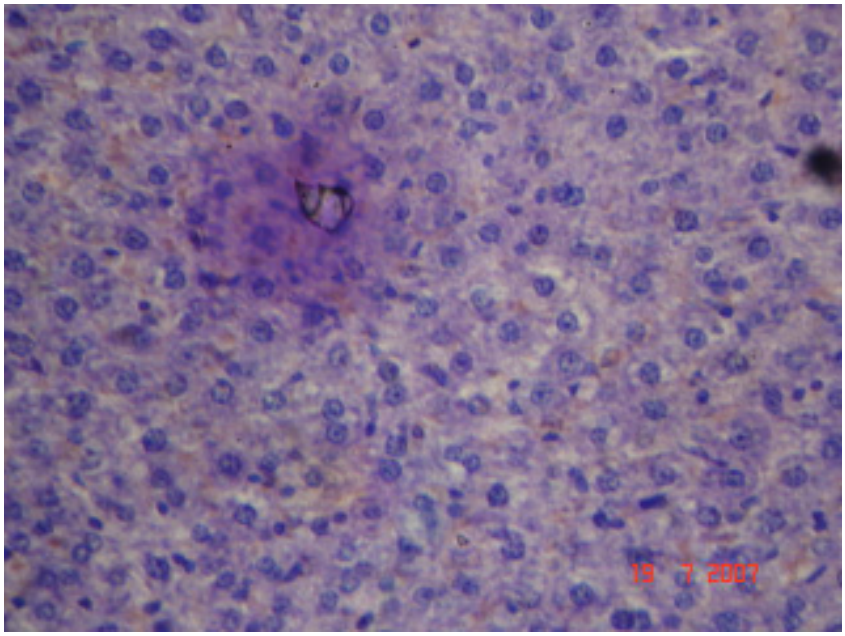


Fig 5 Section of liver tissue of alcoholic extract treated rats showing normal hypatocytes arrangement, absence of necrosis and fatty vacuole around the central vein.

Discussion

The injury and dysfunction of liver caused by CCl_4 in experimental animals simulates the human viral hepatitis [Aoto 1984]. The toxic effect of ccl4 is due to its conversion to highly reactive toxic free radical CCl_3O by cytochrome P450. The free radicals produced locally, because auto oxidation of polyenic fatty acids present within membrane phospholipids and oxidative decomposition of lipid is initiated. The organic peroxides formed after reacting with oxygen leads to swelling of smooth endoplasmic reticulum and dissociation of ribosomes from the rough endoplasmic reticulum. Accumulation of lipids ensues due to inability of the cells too synthesis lipoprotein from triglycerides and lipid acceptor proteins leading to the fatty liver. Further, release of products of lipid per oxidation causes damage to plasma membrane. This is followed by progressive swelling of the cells, massive influx of calcium leading to cell death [Fullbert 1992]. The increase in the levels of AST, ALT, TB and ALP was the clear indication of cellular leakage and loss of functional integrity of the cell membrane [Saraswathi 1993]. Plant constituents like triterpenoids and flavonoids are well known for their antioxidant and hepatoprotective activities [Alex 1997 and Hesham 2002]. Phytochemical analysis of benzene and alcoholic root extracts of *Aeschynomene aspera* revealed the presence of flavonoids, glycosides, triterpenoids and tannin. The concomitant treatment of CCl_4 with root extract showed significant reduction in the level of serum bilirubin and liver function marker enzymes. The test drugs (benzene and alcoholic extracts) mediated restoration in levels of AST, ALT, and ALP towards respective normal value is an indication of stabilization of plasma membranes as well as repair of hepatic tissue due to damage caused by CCl_4. In all the parameters studied, the hepatoprotective activities of BEAA and AEAA were significant as similar to that of silymarin. However the silymarin is slightly effective than these extracts of Aeschynomene *aspera*. Hence,hepatoprotective potency may be attributed to flavonoid, tripenoid present in BEAA and AEAA which is known to normalize the disturbed antioxidant status either by maintaining the levels of glutathione and by inhibiting the production of malondihyde [Martin 2001]. Futher study is warranted to isolate, characterize and screen the active principle from the root of Aeschynomene aspera that possess hepatoprotective properties.

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