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Hepatoprotective activity of the leaf extracts from *Dendrophthoe falcata* (L.f) Ettingsh against carbon tetrachloride –induced toxicity in wistar albino rats

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ABSTRACT

Dendrophthoe falcata (L.f) Ettingsh (Loranthaceae) is a medicinally active, bushy parasitic plant growing on a variety of host plants in the dense forests of India. The present study was conducted to evaluate the hepatoprotective effect of ethanol and aqueous extracts using carbon tetrachloride induced liver damage in wistar albino rats. The study revealed that both ethanol and aqueous extracts significantly reduced AST, ALT, alkaline phosphatase, total bilirubin levels and increased the total protein and albumin levels. These biochemical observations were supplemented by histopathological examination of liver sections. The liver histology of the ethanolic extract treated group showed microfatty changes with a dense collection of lymphoid cells suggesting evidence of very little necrosis or degeneration. The present findings suggest that the leaves of *Dendrophthoe falcata* possess potential hepatoprotective activity. The phenolic compounds and flavonoids in the ethanol extract of leaves of *Dendrophthoe falcata* are responsible for the hepatoprotective activity. The present study scientifically validated the traditional use of *Dendrophthoe falcata* for liver disorders. In conclusion the ethanol extract of *Dendrophthoe falcata* leaves could be an important source of hepatoprotective compounds.

KEYWORDS: Carbon tetrachloride; *Dendrophthoe falcata* (L.f) Ettingsh; Ethanol and aqueous extract; Hepatoprotective activity.

INTRODUCTION

Dendrophthoe falcata (L.f) Ettingsh (Loranthaceae) known as Banda in Hindi, Manda in Oriya and Baramanda in Bengali is a perennial bushy parasitic plant grows in a variety of host plants in deciduous forests through out India (1). The Entire plant is medicinally important and is used in the treatment of impotence, asthma, jaundice, wound, paralysis (2). In previous phytochemical studies, *D. falcata* have been reported to contain several cardiac glycosides, flavonoids and some pentacyclic triterpenes (3, 5). From the literature review, the plant was proved to have antilithiatic, diuretic, cytotoxic and immunomodulatory activities (6, 7). Due to wide spread utilization of the plant as a traditional remedy, it is essential to investigate the potential effects of the crude drug on hepatic tissue for the evaluation of potential health risks in the folklore use.

The hepatitis associated with liver cirrhosis even hepatocellular carcinoma, which has become one of the most prevalent diseases in the world, can be induced by virus, alcohol or other toxic chemicals. Increasing transaminase activities and jaundice were significantly observed in most hepatitis sufferers. Liver damage in animal models can be induced by CCl_4, from which free radical derivatives are biotransformed and lead to increasing lipid peroxidation as well as cell death (8, 9). Therefore, a large amount of transaminases leakage in the blood can be detected, which is often associated with hepatonecrosis (10). In this study, the protective effect of different extracts from *Dendrophthoe falcata* on CCl_4 – induced hepatotoxicity was evaluated through biochemical methods.

MATERIAL AND METHODS

Plant material

Fresh leaves of *Dendrophthoe falcata* were collected in October 2006 from the thick forest areas of Similipal biosphere reservoir, Mayurbhanj district of Orissa. Dr. N. K. Dhal, Sr scientist, RRL, performed taxonomic identification and the voucher specimen was deposited in the herbarium of RRL, (CSIR) Bhubaneswar (vide access no. 9996).

Preparation of extracts

The leaves were washed thoroughly with tap water and air dried in shade at room temperature. They were then mechanically powdered and sieved. 1000gm of

powdered plant material was extracted with ethanolic soxhlation and dried in a rotary evaporator at 40^0C. Another 500gm of the powdered plant material was decocted in a 1000ml of water. The liquid aqueous extract obtained was concentrated in vacuum at 40^0C. The extractive yields were found to be 3.764% and 2.881% for ethanolic (EEDF) and aqueous extract (AEDF) of *Dendrophthoe falcata*, respectively.

Preliminary phytochemical screening

A preliminary phytochemical screening was carried out for the extracts employing the standard procedures to reveal the presence of alkaloids, steroids, terpenes, flavonoids, saponins, tannins, glycosides, carbohydrates and proteins (11).

Animals

Wistar albino rats (100 – 150gm, 50 days old) of either sex were obtained from M/s Ghosh Enterprises, Kolkata. 3 animals were housed per cage (polypropylene cage) and acclimatized for a period of 10 days. Light –dark cycle (light on 6am-6pm) and ambient temperature of 22 ±2 °C and relative humidity of 65% were also maintained on standard pellete diet and water *ad libitum*. The experiment was carried out in between 10.00 to 17.00h. Approval for the study was obtained from the institutional animal ethical committee (IAEC) Reg no.621/02/ac/CPCSEA of Birla Institute of Technology, Mesra.

Acute Toxicity studies

Acute toxicity study was performed for the extract according to the acute toxic classic method as per OECD guidelines (12). Wistar albino rats were used for acute toxicity study. The animals were kept fasting for overnight providing only water, after which the extracts were, administered orally 400mg/kg and observed for 14 days. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If the mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose i.e. 2000mg/kg for each extracts (EEDF, AEDF).

Treatment schedule

The rats were randomly divided into nine groups of six rats each as follows;

Group I (control): Vehicle (0.5% w/v sodium carboxy methyl cellulose in distilled water, 0.2 ml/100g orally) once daily for 7 days.

Group II (induced control): Carbon tetrachloride and olive oil (50% v/v, 0.5ml/kg i.p.) once daily for 7 days (13).

Group III (drug control): Carbon tetrachloride, olive oil and Silymarin (100mg/kg p.o.) simultaneously once daily for 7 days (14, 15).

Group IV, V & VI (Treated): Carbon tetrachloride, olive oil and EEDF (100mg/kg, 200mg/kg & 400mg/kg, respectively) once daily for 7 days.

Group VII, VIII & IX (Treated): Carbon tetrachloride, olive oil and AEDF (100mg/kg, 200mg/kg & 400mg/kg, respectively) once daily for 7 days.

Assessment of liver function

On 8th day the blood was collected by direct cardiac puncture under light ether anaesthesia and serum was separated by centrifuging at 2000 rpm for 15 - 20 min. The serum level of AST and ALT, alkaline phosphatase (16) and total bilirubin (17) was estimated (Table 1). All the tests were carried out with serum diagnostic kits supplied by Span Diagnostic Ltd. Total protein (TP) was estimated by Biuret method (18) where proteins produce a violet color complex with copper ions in an alkali solution and the absorbance of the color complex is directly proportional to the proteins in the sample. While the albumin was estimated by BCG (19) involving formation of blue-green complex with bromocresol green at slightly acidic pH which was measured using standard kits on autoanalyzer 300TX (E-Merck-micro labs, Mumbai).

Histopathological studies

The rats were sacrificed under deep anesthesia and the livers were excised quickly and fixed in 10% buffered neutral formalin. Paraffin sections (5 - 10µ) were prepared, stained with haemotoxylin-eosin, and finally mounted in neutral DPX medium (20). The histopathological examinations were performed using compound microscope.

Statistical analysis

All the data are expressed in Mean ± SEM. One way analysis of variance (ANOVA) followed by benfferoni's *t*-test was applied to compare the significance between all the groups for biochemical parameters. $P < 0.05$ was considered to be significant. All the statistics were estimated by using Sigma Stat 3.5 statistical soft ware (Trial version).

RESULTS

Phytochemical screening

Phytochemical screening for the ethanolic extract of *Dendrophthoe falcata* revealed the presence of phytoconstituents like flavonoids, phenolics, carbohydrates, phytosterols, tannins, volatile oils, fixed oils and proteins. The aqueous extract showed positive results for flavonoids, carbohydrates, proteins and reducing sugar.

Acute toxicity studies

The ethanolic extract and aqueous extract did not cause any mortality up to 2000 mg/kg and were considered as safe.

Carbon tetrachloride induced hepatotoxicity

The results of carbon tetrachloride-induced hepatotoxicity were represented in Table 1. Carbon tetrachloride intoxication in normal rats elevated the levels of AST, ALT, ALP and TB where as decrease levels of TP and ALB were observed significantly indicating acute hepatocellular damage and billiary obstruction. The rats that have received 400 mg/kg of EEDF showed a significant (p<0.05) decrease in all the elevated AST, ALT, ALP, TB levels and significant (p<0.05) increase in reduced TP and ALB levels as compared to induced control group. The rats which have received AEDF showed less significant results.

Normal histology of rat liver shows sinusoidal architecture of hepatocyte having no sign or necrotic degeneration. The liver section of the rats treated with carbon tetrachloride showed cellular degeneration, hydropic changes which were more around the central vein and fatty changes with wide spread hepatocellular necrosis and centrolobular necrosis. The livers of EEDF treated groups showed micro fatty changes with a dense collection of lymphoid cells suggesting evidence of very little necrosis or degeneration. There is no hepatocellular damage except small areas of focal degeneration and sinusoidal dilation in treated rat livers.

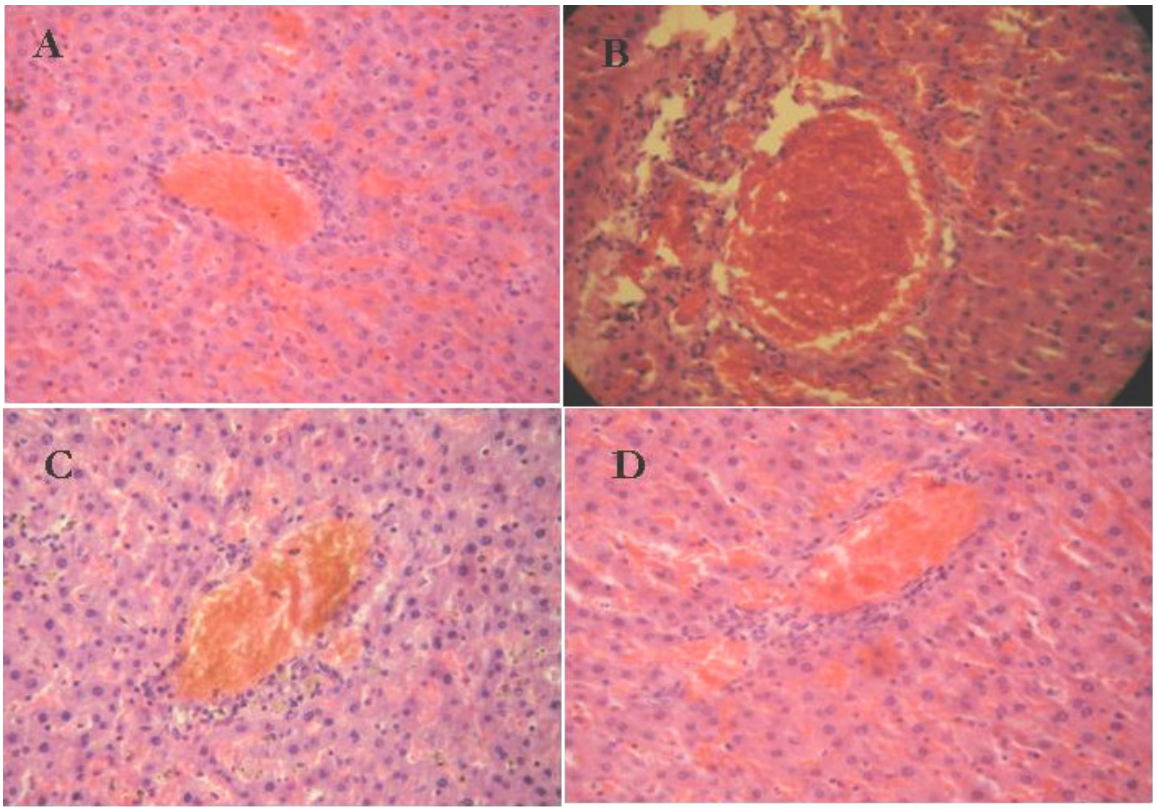

Figure 1: Photographs showing histopathology of liver sections (5-10µ), 400X, Haematoxylin-eosin stain. Liver tissue of, A) Control rat showing normal histology; B)Carbon tetrachloride (0.5ml/kg) treated rat showing cellular degeneration, hydropic changes, fatty changes with wide spread hepatocellular necrosis; C) Silymarin (100mg/kg) treated rat showing very little necrosis or degeneration; D) EEDF (400mg/kg) treated showing no hepatocellular damage except areas of focal degeneration and sinusoidal dilation.

Table 1. Effect of Dendrophthoe falcata on serum enzyme in CCl_4 induced hepatic damage in rats.

Group	Treatment	Serum enzyme levels					
		AST (IU/dl)	ALT (IU/dl)	ALP (IU/dl)	TB (mg/dl)	ALB (g/dl)	TP (g/dl)
I	Vehicle(2ml/kg)	17.47 ±2.84	20.54 ±1.51	41.85 ±2.46	1.58 ±0.46	4.43 ±0.41	7.61 ±0.22
II	CCl_4 (0.5ml/kg i.p.)	38.56 ±4.19	64.12 ±4.89	89.28 ±1.15	4.14 ±0.19	1.82 ±0.22	3.1 ±0.37
III	CCl_4 + Silymarin(100mg/kg)	18.31 ±5.42*	19.40 ±2.80**	43.92 ±7.78**	1.35 ±0.30**	4.81 ±0.38**	6.94 ±0.29**
IV	CCl_4 + EEDF(100mg/kg)	34.17 ±4.22	52.33 ±7.10	78.70 ±2.60	3.39 ±0.44	2.89 ±0.15**	4.22 ±0.34**
V	CCl_4 + EEDF(200mg/kg)	30.88 ±2.49	40.51 ±2.18*	70.66 ±1.89*	2.98 ±0.16*	3.50 ±0.44**	4.91 ±0.38**
VI	CCl_4 + EEDF(400mg/kg)	19.86 ±1.40*	20.33 ±6.50**	46.44 ±5.48**	1.64 ±0.14**	5.14 ±0.29**	6.45 ±0.17**
VII	CCl_4 + AEDF(100mg/kg)	36.81 ±3.11	51.61 ±3.05	85.21 ±4.95	3.88 ±0.48	2.07 ±0.59*	3.73 ±0.35*
VIII	CCl_4 + AEDF(200mg/kg)	34.39 ±6.14	48.14 ±8.44	74.63 ±2.96	3.67 ±0.59	2.23 ±0.33**	4.82 ±0.66**
IX	CCl_4 + AEDF(400mg/kg)	32.31 ±7.23	41.17 ±5.53*	61.97 ±3.39**	3.18 ±1.07*	3.11 ±0.25**	4.47 ±0.34**

*All the data are expressed in mean ± Standard Error Mean from six observations; *p<0.05 and **p<0.001 when compared to CCL_4 treated group (induced control).*

DISCUSSION

The results in the present study indicate that 400mg/kg of EEDF was able to reduce all the elevated biochemical parameters due to the hepatotoxin intoxication. The levels of total proteins and albumin reduced, due to chloralhydrate induced hepatotoxicity. The reduction is attributed to the initial damage produced and localized in the endoplasmic reticulum which results in the loss of p450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver (21). Inhibition of bile acids synthesis in liver or derived from plasma lipids, leading to increase in cholesterol levels was also resulted due to carbon tetrachloride intoxication. The protein and albumin levels were raised suggesting the stabilization of endoplasmic reticulum leading to protein synthesis. The protective effect exhibited by 400mg/kg of EEDF was comparable with standard drug silymarin. The effects at the dose level of 100, 200mg/kg of EEDF and all dose levels of AEDF were not able to alter the elevated parameters caused by carbon tetrachloride intoxication. The histological examination of liver sections reveals that the normal liver architecture was disturbed by hepatotoxin intoxication. In the liver sections of the rats treated with 400mg/kg of EEDF with CCl_4, the normal cellular architecture was retained as compared to silymarin. The results supported the use of this plant for the treatment of hepatitis in oriental traditional medicine.

Flavonoids were reported as active substances for hepatitis induced by chemicals (22) and virus (23) in vitro and in vivo. Ethanolic extract of *D. falcata*, showed positive results for the presence of phenolics and flavonoids during the preliminary phytochemical screening. Also from the literature review, quercetin was found to be the marker compound (4). The possible mechanism may be that the antioxidant potentiality of flavonoids can scavenge free radicals and protect the cell membrane from destruction. Hence, the transaminases (ALT/AST) may not leak into the blood from the necrotic hepatocytes. Further studies aimed at isolating the flavonoids actually responsible for this activity, as well as confirming the observed hepatoprotective effect, are underway in our laboratory.

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