Evaluation of Hepatoprotective Activity of the Stem bark of *Diospyros cordifolia* Roxb.

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Hepatoprotective activity of aqueous and petroleum ether (40-60°) extracts of stem bark of *Diospyros cordifolia* were screened on male Wistar rats against carbon tetrachloride-induced toxic hepatitis. Significant hepatoprotective activity was observed in the petroleum ether extract-treated animals. Biochemical tests representative of liver function such as serum total bilirubin, serum alanine aminotransaminase, aspartate aminotransaminase and alkaline phosphatase activities indicated hepatoprotective activity of the stem bark extracts. Histopathological studies revealed that hepatic lesions occurred in the carbon tetrachloride-treated group, which were reduced significantly by the stem bark extract treatment.

Diospyros cordifolia Roxb. is a deciduous tree, belonging to the family Ebenaceae and popularly known as Indian ebony. In Karnataka State, it is sparsely distributed in Nagarahole National Park, Biligiri Rangan Hills, Kollur Reserve Forests and Bhadra Wild Life Sanctuary[1,2]. The plant is of medicinal importance used for liver disorders whooping cough, leprosy, dysentery, abdominal pains, wounds, gonorrhea, fever, as emetic and anthelmintic[3,4]. The alcoholic extract of the plant possessed antiinflammatory, antipyretic, analgesic[5], CNS depressant and spasmolytic activities in addition to the production of bradycardia and hypotension[6]. Chemical constituents that include, ursolic acid, α-amyrin, β-amyrin, lupeol, taraxerol, hentriacontane, hentriacontanol and β-sitosterol were isolated from leaves of *D. cordifolia* Roxb[7]. Literature survey indicated that evaluation of hepatoprotective activity of stem bark of *D. cordifolia* has not yet been reported so far. This paper reports the hepatoprotective activity of stem bark of *D. cordifolia* against carbontetrachloride-induced hepatic damage in rats.

The stem bark of *D. cordifolia* was collected from the Lakkavalli range forest, Shimoga, Karnataka. The plant was authenticated by comparing with the herbarium specimen at Kuvempu University Herbaria[8] (F.D.D, KUSF-801). The stem bark was shade-dried and powdered mechanically. One kilogram of the powdered material was boiled with distilled water and filtered. Another kilogram of the powdered material was refluxed with petroleum ether (40-60°) in a Soxhlet extractor for 48 h. The extracts were concentrated in vacuum using a flash evaporator (Buchi) and finally dried in a dessiccator.

Oral suspensions (25 mg/ml, w/v) of the extracts were prepared in 2% gum tragacanth. Male Wistar rats (150-200 g) were procured from the central animal house, National College of Pharmacy, Shimoga. The animals were maintained at standard housing conditions and were given food (Hindustan Lever Ltd., Bangalore) and water *ad libitum*. The study was approved by the Institutional Animal Ethical Committee (Reg. No.144/1999/CPCSEA/SMG).

Animals were divided into four groups of six rats each. The animals of group I served as control for 14 d. The animals of the groups II, III and IV received 0.1 ml/kg/day dose of carbon tetrachloride for 14 d intraperitoneally. In addition, the animals of the groups III and IV received orally 25-mg/kg/day dose of each petroleum ether and aqueous extracts, respectively for 14 d[9].

All animals were sacrificed on day 14 under light ether anesthesia. Blood samples were collected by carotid bleeding and allowed to coagulate for 30 min at 37°. Clear serum was separated by centrifugation at 2500 rpm and total bilirubin[10], total protein[11], serum alanine aminotransaminase

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TABLE 1: EFFECT OF STEM BARK EXTRACTS OF *DIOSPYROS CORDIFOLIA* ON CCl_4-INDUCED HEPATOTOXICITY IN RATS.

Group (N)	Total bilirubin mg/dl	Total Protein gm%	AST IU/L	ALT IU/L	ALP IU/L
Control	0.49±0.03	9.25±0.12	51.1±0.61	148.9±0.36	174±2.58
CCl_4-treated	2.39±0.13	5.97±0.32	1390±28.3	2194±42.6	423±18.1
CCl_4+Pet. ether ext.	0.59±0.02*	8.36±0.16*	165±9.81*	241±6.50*	152±6.38*
CCl_4+Aq. ext.	1.02±0.03*	7.29±0.13*	231±9.96*	344±6.13*	230±7.27*

N=6 animals in each group. *P<0.001 indicates significant compared to control. Values are expressed as mean±SEM

(ALT), serum aspartate aminotransaminase[12] (AST) and serum alkaline phosphatase[13] (SALP) activities were determined. Results of biochemical estimations were reported as mean±SEM for determination of significant intergroup differences. Each parameter was analysed separately and was statistically evaluated by Student't' test[14]. The values of p<0.001 were considered as significant. The liver samples were excised from the experimental animals of each group, washed with normal saline. The sections were taken and examined microscopically for the evaluation of histopathological changes.

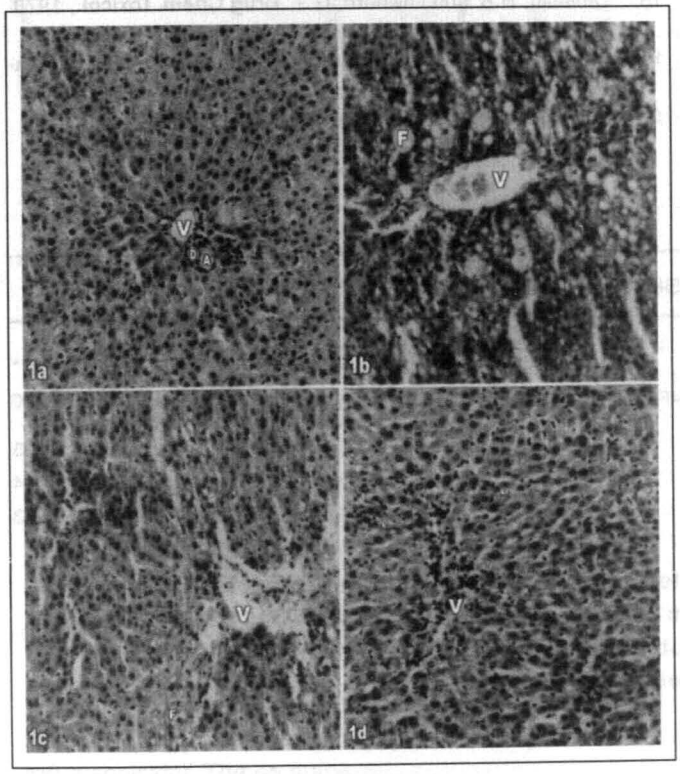

Fig. 1: Histopathology of liver sections.

1a-Liver section of control animal showing normal histology, V-central vein, A-hepatic artery, D-hepatic duct. 1b-Liver section of CCl_4-treated animal showing centrilobular vacuolization, fatty degeneration (F). 1c- Liver tissue of aqueous extract-treated animal showing mild fatty change (F). 1d- Liver section of petroleum ether extract-treated animal showing absence of necrosis, centrilobular vacuolization and fatty degeneration (F).

The hepatotoxicity of CCl_4 begins with the changes in the endoplasmic reticulum, which results in the loss of metabolic enzymes located in the intracellular structures[15]. The toxic metabolite CCl_3 radical was produced by the microsomal oxidase system, which binds covalently to the macromolecules and causes peroxydative degradation of lipid membranes of adipose tissue. In the present study also serum of CCl_4 treated animals showed significant elevation in the levels of liver function biochemical parameters. The elevation in the levels of serum bilirubin is most sensitive and confirms the intensity of jaundice[16] and elevation in the levels of serum enzymes are of indicative of the cellular leakage and loss of functional integrity of the cell membrane[17]. The blood samples of petroleum ether and aqueous extract of *D. cordifolia* treated animals showed significant reduction in the levels of serum markers as shown in the Table 1. Among these the hepatoprotective activity was more in petroleum ether extract treated animals.

The histopathological profile of the normal rat liver showed regular compact arrangement of cells (fig.1a), CCl_4-treated animals showed intense centrilobular necrosis, vauolisation[18] and fatty degeneration (fig.1b). The sections of liver taken from the animals treated with aqueous extract showed moderate accumulation of fatty lobules (fig.1c). While, the liver sections of petroleum ether extract-treated animals showed significant signs of amelioration of liver damage as evident from the normal hepatic cords, absence

of necrosis and fatty infiltration (fig.1d). Further investigations on the isolation and characterization of the active constituents of the above extracts are in progress.

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RP-HPLC Determination of Telmisartan in Tablet Dosage Forms

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A simple fast and precise reverse phase high performance liquid chromatographic method was developed for the determination of telmisartan from tablet dosage forms. A hypersil C18 BDS (250 mm×4.6 mm) from Thermo. In isocratic mode, mobile phase acetonitrile:methanol (60:40) was used. The flow rate was 1.2 ml/min, and eluent monitored at 245 nm.

Telmisartan is 3-N¹-methyl-2-benzimidazole derivative of N¹-4-(2-carboxyphenyl phenyl)-2-propyl, 4-methyl benzimidazole. It is obtained as a white crystalline powder with m.p. 221-223°. It is a new angiotensin II receptor antagonist for the treatment of essential hypertension[1-2]. It is useful in the treatment of mild to moderate hypertension, well tolerated with a lower incidence of cough than ACE inhibitors[3]. It is marketed as 40 mg tablets, taken once daily and in cases where target blood pressure is not achieved dosage could be increased to a maximum of 80 mg once daily[4]. A Milton Roy HPLC system consist of pump CM4000, spectro monitor 3100 of variable wave length detector, chromatography I/F module from Indtech instrument, auto injector A1000 (manual) with 20 micro liter loop and Shimadzu UV-1201 Spectro photometer were used.

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