Evaluation of acute toxicity and hepatoprotective activity of the methanolic extract of *Dichrostachys cinerea* (Wight and Arn.) leaves

P. Suresh Babu, V. Krishna, K. R. Maruthi[1], K. Shankarmurthy, Ramesh K. Babu[2]

Phytochemistry and Pharmacology Laboratory, Department of Post Graduate Studies and Research in Biotechnology and Bioinformatics, School of Biological Sciences, Kuvempu University, Shankaraghatta - 577 451, [1]Department of Post Graduate Studies and Research in Biotechnology, SDM College (Autonomous), Ujire - 574 240, [2]Department of Pathology, Shivamogga Institution of Medical Sciences Shivamogga - 577 201, Karnataka, India

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ABSTRACT

Background: *D. cinerea* are the chief source of drug compounds that are active against various ailments such as jaundice, inflammations rheumatism, fever, asthma, body ache, chest problems, toothache, ulcers, wounds, eye diseases and have an aphrodisiac property. In present study, It was aimed to test the hepatoprotective activity of the plant. **Material and Methods:** The methanolic extract of *Dichrostachys cinerea* (Mimoseae) leaves was subjected to evaluation of acute toxicity and hepatoprotective property, using albino mice and rats. The parameters for estimation of liver function, based on serum markers such as total bilirubin, total protein, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase with histopathological profile of the liver tissue, were studied consequently. **Results:** The methanolic extract did not show any mortality up to a dose of 3500 mg/kg body weight. The methanolic extract showed significant hepatoprotectivity. The histopathological profile of the drug-treated liver tissue demonstrated similar morphology as that of controls. **Conclusions:** Methanolic extract of Dichrostachys cinerea was found to have significant hepatoprotective activity.

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INTRODUCTION

Plants possess broadest spectrum of synthetic activities and are the chief source of many useful compounds. *Dichrostachys cinerea* (Mimosae) is a medium-sized tree commonly distributed in the forests of Africa, Australia, India and parts of Southeast Asia. It is commonly known as "sickle pod", "acacia Saint Domingue", "aroma", in English and its vernacular name is "Vada" in Karnataka, South India. As per the traditional claims, bark and leaves of *D. cinerea* are the chief source of drug compounds that are active against various ailments such as jaundice, inflammations rheumatism, fever, asthma, body ache, chest problems, toothache, ulcers, wounds, eye diseases and have an aphrodisiac property.[1,2] Preliminary phytochemical analysis of the plant, especially leaves and bark extract, proved that it contains flavonoids, tannins, triterpenes, saponins and steroids.[3] The heartwood of *D. cinerea* contains aliphatics and triterpenoids.[4] Antibacterial activity of the tannins isolated from the *D. cinerea* is also well established. In the present investigation, acute toxicity and hepatoprotective property of methanol extract of *D. cinerea* leaves was screened.

Address for correspondence:
Dr. P. Suresh Babu, Phytochemistry and Pharmacology Laboratory, Department of Post Graduate Studies and Research in Biotechnology and Bioinformatics, School of Biological Sciences, Kuvempu University, Shankaraghatta - 577 451, Karnataka, India. E-mail: spbioinfo@yahoo.co.in

MATERIALS AND METHODS

Plant collection and authentication

The fresh leaves of *D. cinerea* were collected from the Malebennur reserve forest range of Karnataka, India. Taxonomic authenticity was confirmed by referring to herbarium specimen at Madras herbarium, Botanical Survey

of India, Southern Circle, Coimbatore, and a voucher specimen (FDD-53) is deposited at Kuvempu University herbaria, Shankaraghatta.

Preparation of extracts

The fresh leaves of *D. cinerea* were shade dried and powdered mechanically. The powdered material was subjected to extraction using soxhlet apparatus with methanol for about 48 hours (200 g × 5). The extract was filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator (Buchi, Flawil, Switzerland) and allowed for complete evaporation of the solvent. The yield of the crude extract crude was 3.16% (w/w).

Preliminary phytochemical investigation

Crude methanolic extract was subjected to preliminary phytochemical qualitative analysis to detect the major chemical groups.[5-7]

Experimental animals

Albino mice of either sex weighing 20–25 g and male Wistar albino rats weighing 180–200 g were procured from Central Animal House, National College of Pharmacy, Shivamogga, and were maintained in standard housing conditions. The animals were fed with commercial diet (Pranav Agro Industries Ltd., Sangli, India) and water *ad libitum* during the experiment. The Institutional Animal Ethical Committee (Reg.No.144/NCP/IAEC/CLEAR/P. COL.3/2006-07) permitted the study to be conducted.

Acute toxicity studies

Per oral (p.o.) acute toxicity of the extract of *D. cinerea* was evaluated in Swiss albino mice, weighing about 20–25 g, by modifying the method of Lorke and Ghosh.[8,9] This method involved the determination of LD_{50} value in a biphasic manner. The animals were starved but allowed access to water 24 hours prior to the study. In the initial investigatory step (phase I), a range of doses of the extract producing the toxic effects was established. This was done by oral administration of widely differing doses of the extract (500, 1000, 2000, 3000 and 4000 mg/kg p.o.) to five groups of mice (four mice in each group). The result showed that there was no mortality up to the dose of 3000 mg/kg b.wt., but maximum mortality was observed at a dose of 4000 mg/kg b.wt. So, a phase II investigatory step was done by giving more specific doses like (3200, 3300, 3400, 3500, 3600 and 3700 mg/ kg p.o.) to five other groups of mice. The mice were observed for 24-48 hours for behavioral signs such as nervousness, excitement, dullness, ataxia or death. Thus, maximum nonlethal dose and the minimum lethal dose were determined as 3500 and 3600 mg/kg b.wt., respectively. One-tenth of the minimum lethal dose at which a minimum 50% of the animal group was mortal (LD-50) was selected as the maximum dose for the evaluation of pharmacological activity of the extracts.[10]

Drug formulations for animal dosage

One-tenth of the minimum lethal dose, i.e., 350 mg/kg b.wt., p.o., was selected as the therapeutic dose for the evaluation of hepatoprotective activity. Drug was prepared in gum tragacanth (1% w/v) in distilled water.

Evaluation of hepatoprotective activity

Animals were divided into four groups with six rats in each group. The animals of group I received the vehicle gum tragacanth (1 ml/kg/day; 1% w/v) and served as control. Carbon tetrachloride with olive oil (1:1) was administered to all the animals of groups II–V in a dose of 0.1 ml/kg/day, intraperitoneally for 14 days.[11] The group III animals treated with the standard drug silymarin (Ranbaxy Lab, Dewas, India; 100 mg/kg/day, p.o.) served as standard. Animals of group IV received methanolic extract (350 mg/kg/day, p.o.). The drugs were administered concomitantly for 14 days. Animals of all the groups were sacrificed on 14th day under light ether anesthesia. The blood sample of each animal was collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 minutes at 37°C. The clear serum was separated by centrifugation at 2500 rpm for 10 minutes and subjected to biochemical investigations for liver function parameters including total bilirubin, total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP).[12]

Statistical analysis

Results of biochemical estimations were expressed as mean ± SEM of all six animals in each group under study. The statistical analysis was carried out using one-way analysis of variance (ANOVA) on the data, followed by Dunnett's *t*-test. The difference in values at $P \leq 0.01$ was considered as statistically significant.

Histopathologic study

The liver was excised from all the six animals of each group separately after draining the blood and was washed using normal saline. Initially, the liver of each animal was fixed in 10% buffered neutral formalin for 48 hours. Further, the liver of each animal was processed by paraffin embedding. Sections of 5 μm thickness were taken, processed in alcohol-xylene series and stained with alum-hematoxylin and eosin. The sections were examined microscopically for the evaluation of histopathologic changes.[13,14]

RESULTS

The qualitative phytochemical analysis of methanolic extract of *D. cinerea* showed the presence of alkaloids,

sterols, triterpenes, glycosides, tannins and proteins. Methanolic extract did not show any sign or symptom of toxicity and mortality up to a dose of 3500 mg/kg.

Evaluation of hepatoprotective activity

After 14 days treatment, biochemical analysis of blood samples of CCl_4 treated animals showed significant increase in the levels of total bilirubin (6.22-fold), AST (5.71-fold), ALT (4.22-fold) and ALP (3.47-fold), as compared to the controls. In addition, the total protein level (44.86%) was decreased, reflecting the liver injury due to the toxic effect of CCl_4. The blood samples of the animals treated with methanolic extract showed significant reduction in the levels of liver function serum markers. The effect was more pronounced in the animals treated with methanol extract, thus proving the significant hepatoprotective action of the extract [Table 1].

Histopathologic observations

The histopathologic profile of control animals showed a normal architecture of hepatocytes [Figure 1a]. The histological observations of liver sections of animals treated with CCl_4 revealed intense centrilobular necrosis, vacuolization and macrovesicular fatty changes [Figure 1b]. The liver sections of silymarin treated animals showed normal tissue architecture [Figure 1c]. Normal hepatic cords with moderate fatty infiltration was observed in the liver sections of methanolic extract treated animals, demonstrating significant liver protection against CCl_4-induced liver damage, as is evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration with reformation of tissue architecture [Figure 1d].

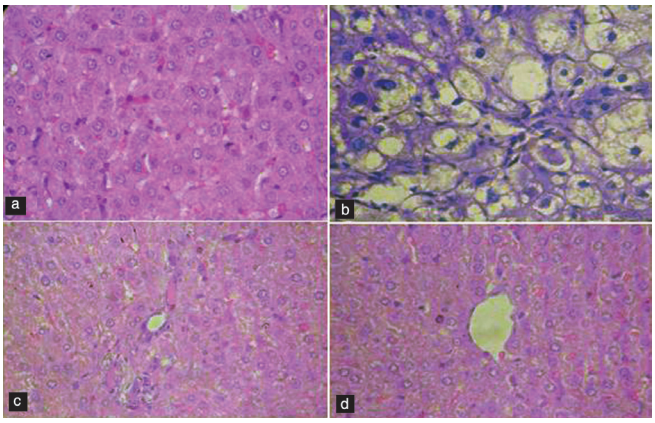

Figure 1: (a) Histology of the liver sections of control animals (Group I) showed normal hepatic cells with well-preserved cytoplasm, prominent nucleus, nucleolus and visible central veins; (b) the liver sections of CCl4-intoxicated rats exhibited intense centrilobular necrosis, vacuolization, macrovesicular fatty changes showing massive fatty accumulation in the hepatocytes, and broad infiltration of the lymphocytes and the loss of cellular boundaries; (c) the histopathologic observations of liver tissue section of silymarin treated group showed regeneration of cells with compact arrangement and lack of fatty lobulation; (d) the histological architecture of liver sections of the rats treated with methanol extract of *D. cinerea* exhibited significant liver protection against CCl4 injury as evident by the presence of normal hepatic cords, absence of necrosis, fatty infiltration

DISCUSSION

Medicinal plants are an integral component of research and development in pharmaceuticals. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs from plants as well as from traditionally used rural herbal remedies. The present investigation revealed the hepatoprotective efficacy methanolic extract of the *D. cinerea* leaves in CCl_4-induced hepatic damage in experimental rat models.

It is well established that CCl_4 induces hepatotoxicity by metabolic activation. Therefore, it selectively causes toxicity in liver cells, maintaining semi-normal metabolic function.[15] CCl_4 is bio-transformed by the cytochrome P450 system in the endoplasmic reticulum to produce trichloromethyl free radical ($\bullet CCl_3$). Trichloromethyl free radical, when combined with cellular lipids and proteins in the presence of oxygen, forms trichloromethyl peroxyl radical which may degrade lipids on the membrane of endoplasmic reticulum, and thus elicits lipid peroxidation and finally results in cell death.[16] In this present study, it was observed that the administration of CCl_4 decreased the levels of total protein and increased the levels of marker enzymes. The estimation of total bilirubin depicts the depth of jaundice and the elevated level of liver function serum marker enzymes, viz., ALT, AST and ALP, indicates the intensity of liver damage. Marked elevation in the levels of total bilirubin and marker enzymes in the serum of CCl_4-intoxicated rats was observed. But measurable decrease of the serum marker levels was

Table 1: Hepatoprotective activity of the methanolic extract of the *D. cinerea* leaves

Samples	Total bilirubin (mg/dl)	Total protein (gm/dl)	AST (IU/l)	ALT (IU/l)	ALP (IU/l)
Control	0.36 ± 0.08	7.40 ± 0.36	209.41 ± 13.57	76.31 ± 9.11	116.86 ± 9.57
CCl_4	2.24 ± 0.29	4.08 ± 0.27	1194.08 ± 73.6	321.67 ± 27.93	403.73 ± 8.66
Silymarin + CCl_4	0.40 ± 0.14*	7.34 ± 0.42*	217.64 ± 23.8*	78.81 ± 8.05*	118.21 ± 9.7*
Methanol crude + CCl_4	0.52 ± 0.09*	7.33 ± 0.23*	289.49 ± 22.66*	112.27 ± 4.61*	169.74 ± 19.85*

Values are expressed as mean ± SEM of six samples. Data were analyzed by one-way ANOVA followed by Dunnett's *t*-test. *represents $P < 0.01$ when compared to control

observed in the serum of animals treated with methanolic extract. The protection against the injurious effects of CCl_4, which may result from the interference with cytochrome P450, leads to the hindrance of the formation of hepatotoxic free radicals. The site-specific oxidative damage in some susceptible amino acids of proteins may be regarded as the major cause of metabolic dysfunction of liver during pathogenesis (Uday *et al.,* 1999). The restoration of bilirubin levels may be due to the inhibitory effects on cytochrome P450 or/and promotion of its glucuronidation.[17] The attainment of these marker enzymes toward a near-normalcy in the animals treated with methanol extract proved the hepatoprotective effect of the extract.

CONCLUSION

It can be said that the methanolic extract of *D. cinerea* has afforded protection against CCl_4-induced liver damage. The mechanism by means of which the extract may protect the liver from CCl_4 toxicity or may act as a free radical scavenger intercepting those radicals involved in CCl_4 metabolism is by its action on microsomal enzymes. By trapping oxygen-related free radicals, the compound would hinder their interaction with polyunsaturated fatty acids and abolish the enhancement of lipid peroxidative processes. The present investigation also supports the ethnomedical uses of *D. cinerea* and encourages further research on this plant.

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