HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF *Boerrhaviadiffusa* L. AGAINST CARBON TETRACHLORIDE (CCL_4) INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Objective of the present study was to assess the hepatoprotective activity of the methanolic extract of *Boerrhavia diffusa* L. against CCl_4 induced hepatotoxicity in rats. CCl_4 was administered @2.5 mg/kg as 1:1 in olive oil. The methanolic extract of B.diffusa (whole plant)was administered in the dose rates of 250 and 500 mg/kg orally at 12h intervals and after 36h., blood was collected, serum separated and biochemical parameters like SGOT, SGPT, ALP, total bilirubin and direct bilirubin were estimated.The liver samples were subjected to histopathological studies. Treatment with the extract significantly reduced the liver damage induced by CCl_4 as indicated by a decrease in the elevated levels ofall the biochemical parameters to near normal values in a dose-dependantmanner. The histopathological findings were also complimentary to these results. Themethanolic extract of *B. diffusa* possessed significant protective activity against CCl_4 induced liver toxicity in rats.

Keywords: *Boerrhaviadiffusa* L, hepato toxicity, carbon tetrachloride

INTRODUCTION

Boerrhavia diffusa L. commonly known as "punarnava" in Sanskrit and 'spreading hogweed' in English has been used in traditional Ayurvedic medicines alone and in combination with other plants for the treatment of liver, gall bladder, renal and urinary disorders since ages. The earliest mention of this plant is seen in Charaka Samhita (Charaka, 1949). The plant was included as one of the extensively investigated medicinal plants in India by Vohora (1989). Abraham (1979) reported that the plant as a whole was effective in jaundice, oedema and blood pressure. Two known lignansureodendrin and syringarexual mono-β-D glucoside have been isolated from the methanolic extract of the roots of the plant (Lami *et al*., 1991) and the former was found to exhibit Ca channel antagonistic effect. Singh *et al*. (1991) observed that the ethanolic extract of *B. diffusa* @ 250 mg/kg did not have any teratogenic effect. Rawar *et al*. (1997) investigated the effect of seasons and thickness of roots on the hepatoprotective effect of the plant against thioacetamide induced hepatotoxicity in rats. They found that an aqueous extract of roots of diameter 1-3 cm collected during summer (May) exhibited maximum protection of the serum enzymes SGOT, SGPT and ALP.

An earlier study by the same author has revealed that the methanolic extract @ 200 mg/kg body weight possess significant anti-inflammatory activity both acute and chronic inflammatory models in rats. The present study was conducted to find out the effect of methanolic extract of *B. diffusa* on CCl_4

induced hepatotoxicity in rats.

MATERIALS AND METHODS

Chemicals

CCl_4 was purchased from Merck India Ltd. The auto analyser kits for estimation of serum ALT, AST, AP, TB and DB were purchased from Merck India Ltd.

Plant material

The whole plant of *B. diffusa* L. were collected from the premises of Veterinary College, Mannuthy and authenticated at Medicinal Plants Division of Horticultural College of KAU.

Animals

Wistar albino rats weighing around 150-200 g of either sex procured from SABS, Mannuthy were used for the study. They were housed in well ventilated cages (temperature 30 ± 2°C, humidity 65-70% and 12 h light/ dark cycle) and fed with standard rodent diet from SABS, Mannuthy and drinking water ad libitum. Animal studies were conducted according to the Ethics Committee regulations of COVAS, Mannuthy.

Extraction

The plant materials (whole plant including leaves, stem and roots) were dried under shade at 30°C, pulverized and extracted with methanol in a soxhlet apparatus. The extract was evaporated to dryness under reduced pressure.

CCl_4 induced hepatotoxicity

Rats were divided into 4 groups of 8 each. Group I (control) was administered a suspension of distilled water and olive oil (1:1 proportion). Group II served as toxic control and received 1:1 mixture of CCl_4 suspension in olive oil @ 2.5 ml/kg bodyweight. Group III and IV were treated with methanolic extract of *B. diffusa* @ 250 and 500 mg/kg po. All the doses were repeated at 12 hours and 24 hours respectively. 36 hours after the initial dose, the animals were sacrificed under light ether anaesthesia. Blood was collected from the retro-orbital plexus of all the rats and allowed to clot for 30 minutes at 37°C. Serum was separated by centrifugation at 2500 rpm at 37°C for 15 minutes and analysed for SGOT, SGPT, ALT, TB and DB (Bergmeyer, 1980 and Perry *et al*., 1986).

Histopathological studies

After draining the blood, liver samples were excised, washed with normal saline and processed separately for histopathological studies. Initially, the material was fixed on 10% buffered neutral formation for 48 hours. The sections were then dehydrated in gradual ethanol (50-100%) cleared in xylene and embedded in paraffin sections (4-5 µm thick) were prepared and stained with hematoxylin and eosin dye for photomicroscopic examination.

Statistical Analysis

All the data are expressed as mean ± SEM. One way analysis of variance was used for the statistical analysis of data. Students' t test (Woolson, 1987) was used to determine the significance. The probability value of $P<0.05$ was considered as significant.

RESULTS

The levels of all the serum parameters studied were increased significantly in the toxicant group ie., Group II (Table 1). The levels of AST, ALT, total and direct bilirubin showed significant decrease in the group IV when compared with the toxicant Group i.e., Group II. The levels of AP and TB were also decreased, though not significantly. Similarly the levels of all the parameters except AP were decreased in the Group III also, though not significantly.

The results of histopathological study can be read from the Fig. 1, 2, 3 and 4.which correspond to the groups I , II , III and IV respectively.

Table. 1. Assessing the hepatoprotective activity of the methanolic extract- results of biochemical study

Group No	Treatment	ALT (U/l) _ SE)	AST (U/l) (Mean ± SE)	AP (U/l) (Mean ± SE)	TB (mg/dl) (Mean ± SE)	DB (mg/dl) (Mean ± SE)
I	Vehicle	209.5 ± 21.85	73.25 ± 11.97	310 ± 46.99	0.34 ± 0.5	0.28 ± 0.5
II	Toxicant	1150.86 ± 281.68	3405.86 ± 739.72	1375.25 ± 281.0	1.81 ± 0.37	1.2 ± 0.4
III	Ext @ 200 mg/kg	696.375 ± 41.067	607.125 ± 198.827	1392.25 ± 226.53	1.8 ± 2.8	0.963 ± 0.18
IV	Ext @ 400 mg/kg	78.375 ± 10.48*	425.5 ± 70.22*	1025.85 ± 140.99	0.713 ± 1.2	0.5125 ± 0.16*

*Significant at 5% level

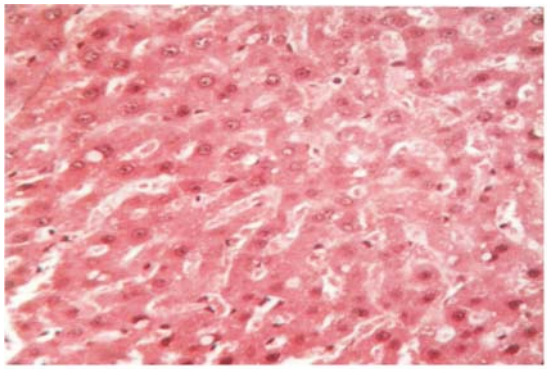

Fig. 1 The hepatic cells are radially placed and each cell has a large spherical nucleus with pronounced nucleolus and granular cytoplasm

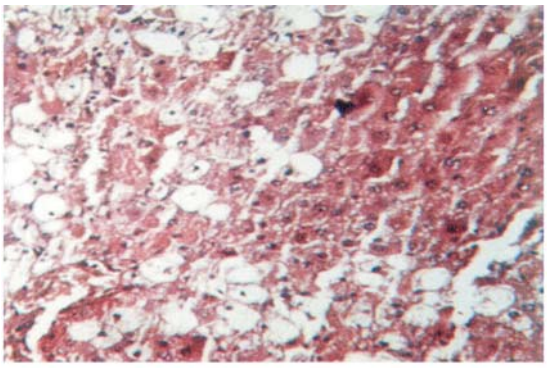

Fig. 3. Hepatic cells have become more distend with prominent nucleus and are arranged in the form of cords. Vacuolation also is lessened.

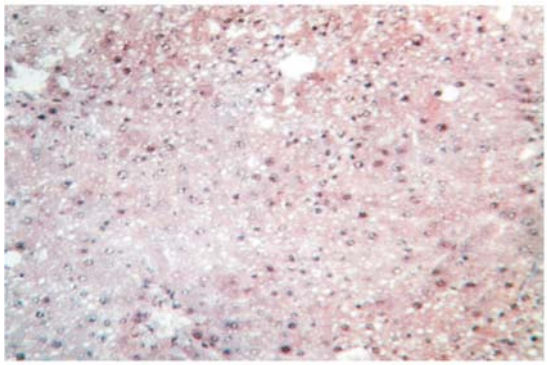

Fig.2 There is heavy destruction of the overall arrangement of liver cells because most of the cells are in a ruptured state and without cytoplasm. Space formation and high degree of vacuolation are also seen.

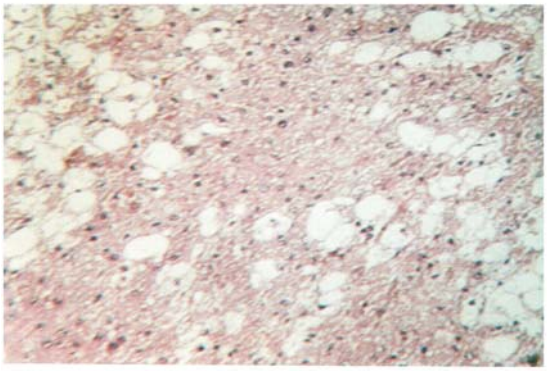

Fig.4. The liver section is almost normal, with minimum vacuolation and clear hepatic cells.

DISCUSSION

The results suggest that the methanolic extract of B. diffusa @ 400 mg/kg possess significant hepatoprotective activity. CCl_4 produces hepatotoxicity by metabolic oxidation. It is transformed by the cytochrome P450 system to produce trichloromethyl radical and trichloromethylperoxy radical which are responsible for the oxidative degradation in the adipose tissue resulting in fatty infiltration, destruction of Ca2+ homoeostasis and cell death (Clawson, 1989). As a result, there will be leakage of marker enzymes like SGOT, SGPT and ALP in the serum and increase in serum TB and DB levels (Recnagel, 1989).

The plant *B. diffusa* has been found to contain a variety of phytochemicals i.e flavors like 5-7 dehydroxy 3'-4' dimethoxy 6-8-dimethyl flavors, reducing engars, tritenpenes like B-sietosterol, alkaloids like punarnavine, tannins, amino acids like alanine, aspartic acid, methionine, threonine and histidine and lignins like lignodendrin (Asolkar *et al*., 1992). According to Heinrich *et al*. (1998) flavones bind to enzymes and cell membranes and complex heavy metal ions, participate in the electron transfer of enzyme systems and exhibit free radical scavenging activity. Similarly, the hepatoprotectiveactivity of many flavoids have been reported by Rajnarayana *et al*. (2001). The results are also complementary to the findings of Rawal *et al*. (1997) who found that the roots of B. diffusa have significant protective activity against thioacetamide induced hepato toxicity in rats. The flavanoids and other constitutents may be responsible for the hepatoprotective activity of the plant. The isolation and characterization of the flavanoids and other constituents and their pharmacological screening need detailed trials.

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