

Hepatoprotective Properties of *Bauhinia variegata* Bark Extract

Surendra H. BODAKHE* and Alpana RAM

SLT Institute of Pharmaceutical Sciences, G. G. University, Bilaspur (C.G.)–495009, India

(Received March 16, 2007; Accepted June 4, 2007)

Bauhinia variegata (Leguminosae) commonly known as Kachnar, is widely used in Ayurveda as tonic to the liver. The present work was carried out to assess the potential of *Bauhinia variegata* bark as hepatoprotective agent. The hepatoprotective activity was investigated in carbon tetrachloride (CCl₄) intoxicated *Sprague-Dawley* rats. *Bauhinia variegata* alcoholic Stem Bark Extract (SBE) at different doses (100 and 200 mg/kg) were administered orally to male *Sprague-Dawley* rats weighing between 100–120 g. The effect of SBE on the serum marker enzymes, viz., AST, ALT, ALP and GGT and liver protein and lipids were assessed. The extract exhibited significant hepatoprotective activity. Hence, *B. variegata* appears to be a promising hepatoprotective agent.

Key words—*B. variegata*; carbon tetrachloride; hepatoprotective; marker enzyme

INTRODUCTION

Liver is the major site of intense metabolic activities. The role played by this organ in the removal of substances from the portal circulation makes it susceptible to first and persistent attack by offending foreign compounds culminating in liver dysfunction. Herbal medicines have been the oldest forms of healthcare. In Ayurveda many indigenous plants have been mentioned and well established as hepatoprotective agents. Yet there is a paucity of information regarding the activity of *Bauhinia variegata* in liver diseases. This study was undertaken to fill the lacuna in this regard.

Bauhinia variegata (Leguminosae) is a medium sized deciduous tree. The bark is astringent to the bowel and tonic to the liver.¹⁾ Various flavone glycosides have been isolated from the seeds and roots of *Bauhinia variegata*.^{2,3)} The flavonoid isolated from various parts has been reported as effective hypoglycemic agent.⁴⁾ The seed proteins exhibited haemagglutinating effect.⁵⁾ The methanolic extract of the leaves of *B. variegata* has been reported to have antibacterial and antifungal effects.⁶⁾

A number of pharmacological and chemical agents act as hepatotoxin and produce variety of liver ailments.⁷⁾ Our experiment was designed to use carbon tetrachloride (CCl₄) intoxicated rat liver as model. The procedure, technique and biochemical es-

timations were carried by using the method of Venkumar and Latha 2002.⁸⁾

EXPERIMENTAL

Preparation of Plant Extract Stem bark of *Bauhinia variegata* Linn was collected from G. G. University campus in October 2005 and was authenticated by plant taxonomist Dr. C. Rajasekharan. A voucher specimen (SLT-Med. Plant. –721) was deposited in the S.L.T. Institute of Pharmaceutical Sciences, Guru Ghasidas University, Bilaspur (Chhattisgarh, India). Stem bark were separated from wood and used for extraction. The collected materials were washed thoroughly in water, chopped, air dried for a week at 35–40°C and pulverized in electric grinder. The powder obtained was successively extracted with alcohol. The extract was concentrated under reduced pressure and dried.

Experimental Animals Male *Sprague-Dawley* rats weighing between 100–120 g were used in present study and were purchased from CCS Haryana Agriculture University, Hisar (Haryana, India). The animals had free access to food and water and were maintained under controlled temperature (27±2°C) and 12 h: 12 h light and dark cycle. Initial body weight of each animal was recorded.

Acute Toxicity Studies *Bauhinia variegata* stem bark extract (SBE) at different doses (50–2000 mg/kg) was administered orally to normal rats. During the first four hours after the drug administration, the animals were observed for gross behavioral changes if

*e-mail: bodyas@rediffmail.com

any for 7 days. The parameters such as hyperactivity, grooming, convulsions, sedation, hypothermia, mortality were observed and doses selected were 100 and 200 mg/kg. Institutional Animal Ethical Committee (IAEC) had approved the experimental protocol and care of animals was taken as per the guidelines of CPCSEA, Department of Animal Welfare, Government of India.

Experimental Design Liver damage was induced in rats by administering CCl_4 subcutaneously (*s.c.*) in the lower abdomen in a suspension of liquid paraffin (LP) in the ratio 1 : 2 v/v at the dose of 1 ml CCl_4 /kg body weight of each animal. CCl_4 was administered twice a week, on every first and fourth day of all the 13 weeks.

Thirty-two rats were divided into 4 groups of 8 animals each as follows:

Group I animals served as control and received *s.c.* administration of LP only at the dose of 3 ml/kg body weight, twice a week for a duration of 13 weeks (89 days). Group II animals received *s.c.* administration of LP + CCl_4 twice a week for a total of 13 weeks. Groups III and IV animals were the SBE-treated animals and received *s.c.* administration of LP + CCl_4 as in Group II rats, besides they received orally a SBE suspension of 1 ml water at the dose of 100 and 200 mg/kg body weight daily for 13 weeks respectively. Replenishing a known quantity of fresh food daily at 8.00 a.m. and thereby measuring the food intake of the previous day carried out measurement of daily food consumption. Body weight of rats was recorded weekly to assess percentage of weight gain of each animal. Animals were kept starved overnight on the 89th day. On the next day, after recording the weight of each animal, they were sacrificed by decapitation by making an incision on jugular vein to collect blood. The liver tissue was dissected out, blotted off blood, washed in saline and weighed instantaneously. This was kept in frozen containers and proceeded for biochemical estimations.

Biochemical Estimations Serum was prepared from the collected blood and subjected to biochemical estimations of different parameters like aspartate aminotransferase, AST,⁹⁾ alanine aminotransferase, ALT,⁹⁾ alkaline phosphatase, ALP,¹⁰⁾ gamma glutamyl transpeptidase, GGT,¹¹⁾ total proteins¹²⁾ and total lipids.¹³⁾ Liver homogenates were also subjected to various biochemical estimations.

Histopathology A portion of liver tissue in

each group was fixed in 10% formalin (formalin diluted to 10% with normal saline) and proceeded for histopathology. Sections were stained with Ehrlich's hematoxylin and eosin.

Statistical Analysis One-way analysis of variance (ANOVA) was applied for determining the statistical significance of difference in serum marker enzymes, protein and lipid levels between different groups. Results were considered statistically significant at $p < 0.05$.

RESULTS

Acute Toxicity Studies No mortality observed with oral administration of SBE even at the highest dose (2000 mg/kg).

Effect on Food Consumption and Weight Gain The food consumption and weight gain significantly increased in Ggroups III and IV animals as compared to Group II.

Effect on Biochemical Parameters All the marker enzymes, *viz.*, AST, ALT, ALP and GGT registered enhanced activity in Group II rats as compared to Group I. In Group III and Group IV, the levels of these enzymes were found retrieving towards normalcy (Fig. 1). The results are presented in Table 1 and 2. The total protein concentration of the serum and liver was lesser in Group II animals and it attained an almost normal value in Group III and IV rats (Figs. 2 and 3). The total lipid concentration of

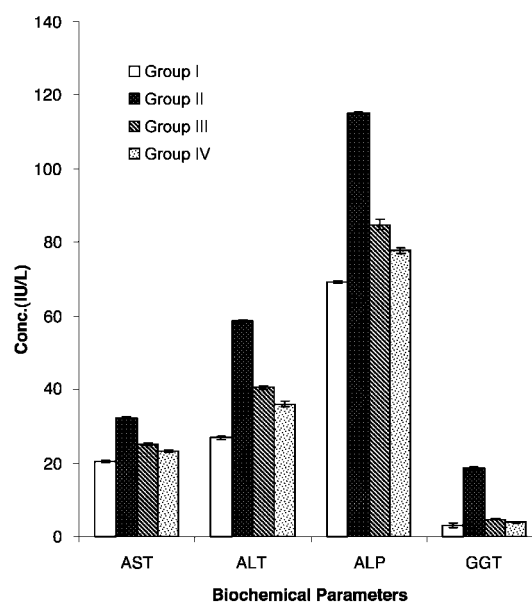


Fig. 1. Effect of Alcoholic Stem Bark Extract on Serum AST, ALT, ALP, GGT

Table 1. Effect of Alcoholic Stem Bark Extract (SBE) on Biochemical Parameters in Serum

Treatment	Biochemical parameters ^{a)}					
	AST (IU/l)	ALT (IU/l)	ALP (IU/l)	GGT (IU/l)	Total protein (mg/dl)	Total lipids (mg/dl)
Group I (Control, LP only)	20.47±0.27	26.86±0.38	69.18±0.42	3.11±0.67	5.63±0.15	131.11±1.27
Group II (LP+CCl ₄)	32.21±0.33 ^{b)}	58.68±0.43 ^{b)}	115.18±0.42 ^{b)}	18.56±0.50 ^{b)}	3.9 ±0.22 ^{b)}	259.38±3.56 ^{b)}
Group III (LP+CCl ₄ +SBE 100 mg/kg)	25.05±0.34 ^{c)}	40.59±0.54 ^{c)}	84.88±1.33 ^{c)}	4.73±0.20 ^{c)}	4.76±0.24 ^{c)}	164.51±6.08 ^{c)}
Group IV (LP+CCl ₄ +SBE 200 mg/kg)	23.28±0.33 ^{c)}	36.15±0.67 ^{c)}	77.77±0.87 ^{c)}	3.93±0.11 ^{c)}	5.4 ±0.18 ^{c)}	134.68±1.67 ^{c)}

a) Values are mean±SEM of 8 animals in each group, b) p<0.05 as compared to Group I, c) p<0.05 as compared to Group II.

Table 2. Effect of Alcoholic Stem Bark Extract (SBE) on Biochemical Parameters in Liver

Treatment	Biochemical parameters ^{a)}	
	Total protein (g/100 g)	Total lipids (mg/100 g)
Group I (Control, LP only)	8.11±0.12	6.13±0.22
Group II (LP+CCl ₄)	5.72±0.13 ^{b)}	8.08±0.14 ^{b)}
Group III (LP+CCl ₄ +SBE 100 mg/kg)	6.86±0.26 ^{c)}	7.2±0.23 ^{c)}
Group IV (LP+CCl ₄ +SBE 200 mg/kg)	7.77±0.21 ^{c)}	6.96±0.23 ^{c)}

a) Values are mean±SEM of 8 animals in each group, b) p<0.05 as compared to Group I, c) p<0.05 as compared to Group II.

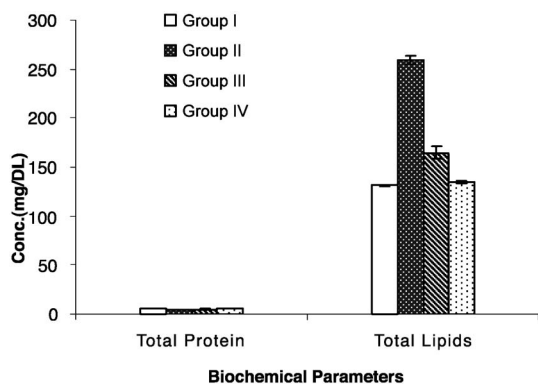


Fig. 2. Effect of Alcoholic Stem Bark Extract on Total Proteins and Total Lipids in Serum

the serum and liver was high in Group II animals and it attained an almost normal value in Groups III and IV rats (Figs. 2 and 4).

Effect on Liver Histopathology Histopathological study of liver from Group I animals showed a

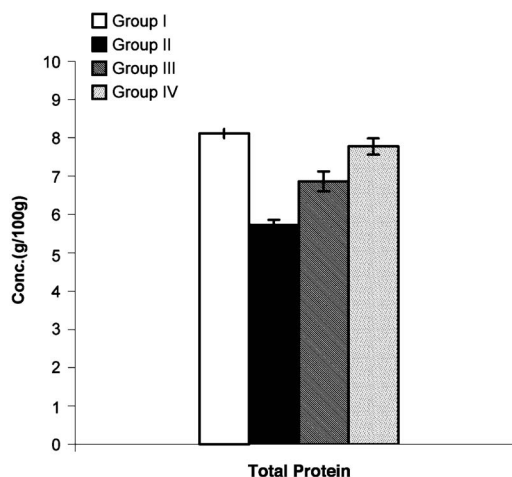


Fig. 3. Effect of Alcoholic Stem Bark Extract on Total Proteins in Liver

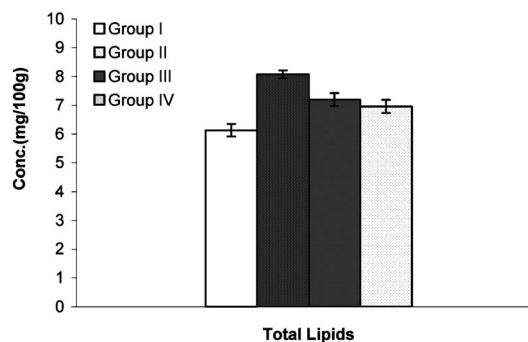


Fig. 4. Effect of Alcoholic Stem Bark Extract on Total Lipids in Liver

normal hepatic architecture (Fig. 5). In Group II, severe hepatotoxicity was seen (Fig. 6). In Groups III and IV animals the liver exhibited an almost normal

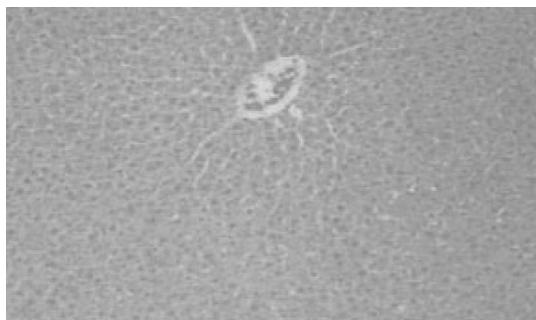


Fig. 5. Photomicrograph of Liver Section from Group I Rats Showing Normal Hepatic Architecture

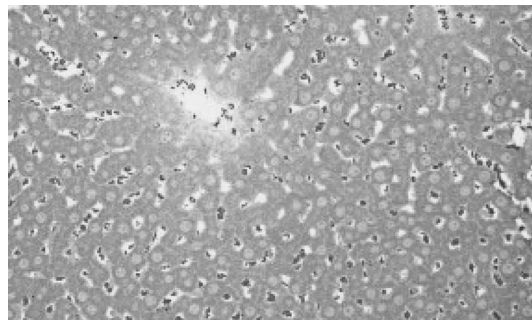


Fig. 7. Photomicrograph of Liver Section from Group III Rats Showing almost Normal Architecture

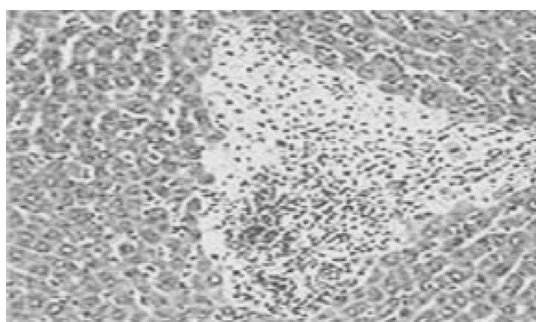


Fig. 6. Photomicrograph of Liver Section from Group II Rats Showing Severe Hepatotoxicity

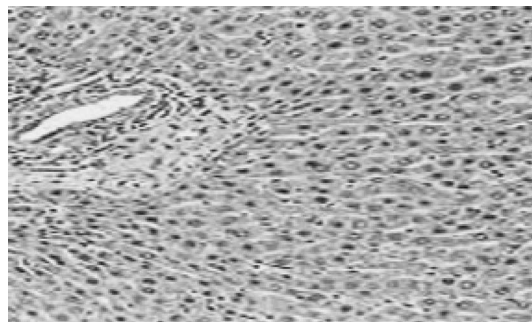


Fig. 8. Photomicrograph of Liver Section from Group IV Rats Showing almost Normal Architecture

architecture (Figs. 7 and 8).

DISCUSSION

Carbon tetrachloride is commonly used as standard hepatotoxin.¹⁴⁾ It is converted by the liver drug metabolizing enzyme system into CCl_3 radical, which attacks unsaturated fatty acids of membranes in the presence of oxygen to give lipid peroxides. Consequently, the functional integrity of the hepatic mitochondria is altered. All these events ultimately lead to liver damage.¹⁵⁾ The enhanced activities of these serum marker enzymes observed in CCl_4 treated rats correspond to the extensive liver damage induced by the CCl_4 . Estimating the activities of serum marker enzymes, like AST, ALT, ALP and GGT, can make assessment of liver function. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage.¹⁶⁾ In this context we have also observed a rise in the levels of AST, ALT, ALP and GGT in carbon tetrachloride treated rats. Return of these enzyme levels towards normal level in Groups III and IV rats is a clear indication of hepatoprotective effect of SBE. In the present study

SBE seems to offer protection and maintain the structural integrity of hepatic cells. Hepatotoxins increase the levels of total lipids in liver.¹⁷⁾ Total lipid content in serum and liver registered a significant hike, which was retrieved to near normalcy in SBE treated rats. This is the clear indication of the improvement of the functional integrity of the liver cells. Carbon tetrachloride impairs the capacity of the liver to synthesize albumin.¹⁸⁾ So the protein content of serum decreases in such cases. Retrieval of protein concentration to normalcy further confirms *Bauhinia variegata* Stem bark extracts hepatoprotective effect and its use as liver tonic. The protective effects were more pronounced and much better in Group IV than Group III rats, i.e. SBE at 200 mg/kg dose showed better anti-hepatotoxic activity as compared to 100 mg/kg dose, against carbon tetrachloride induced hepatic damage. On the basis of above results it could be concluded that the hepatoprotective effect increased with increase in the dose.

However more elaborate work is required to establish the efficacy of SBE as potent anti-hepatotoxic drug. Further experimental work is necessary to iso-

late and identify the active principles present in the SBE that are responsible for the anti-hepatotoxic activity.

Acknowledgements Authors are deeply grateful to the SLT Institute of Pharmaceutical Sciences, G. G. University, Bilaspur (C.G.)-495009 and SGRR Institute of Technology & Science, Patel Nagar, Dehradun (India) for research facilities. The authors deeply appreciate the cooperation and helpful criticism extended by Munish Ahuja, Lecturer, Department of Pharm. Sciences, Guru Jambheshwar University, Hisar (Haryana, India).

REFERENCES

- 1) Kirtikar K. R., Basu B. D., "Indian Medicinal Plants," Vol. III, Oriental Enterprises, Dehradun, 1257-1260 (2001).
- 2) Yadava R. N., Reddy V. M. S., *J. Asian Nat. Prod. Res.*, **4**, 341-346 (2001).
- 3) Yadava R. N., Reddy V. M. S., *Nat. Prod. Res.*, **3**, 165-169 (2003).
- 4) El-Wahab A., Wassel S. M., Amar G. M., Hanna T., *Herb. Hung.*, **1**, 27-39 (1987).
- 5) Wassel G., El-Wahab S. A., Amar N., *Herb. Hung.*, **2**, 123-125 (1989).
- 6) Sharma R. N., Saxena V. K., *Asian J. Chem.*, **4**, 811-812 (1996).
- 7) Ram V. J., Goel A., *Curr. Med. Chem.*, **6**, 217-254 (1999).
- 8) Venukumar M. R., Latha M. S., *Ind. J. Pharmacol.*, **34**, 269-275 (2002).
- 9) Reitman S., Frankel A. S., *Am. J. Clin. Pathol.*, **28**, 53-56 (1957).
- 10) Kind P. R. N., King A. J., *J. Clin. Pathol.*, **7**, 322-326 (1954).
- 11) Naftalin L., Markaret S., Whitaker J. F., Tracey D., *Clin. Chem. Acta*, **26**, 293-296 (1969).
- 12) Lowry O. H., Rosebrough N. J., Farr A. L., Randall R. J., *J. Biol. Chem.*, **193**, 265-275 (1951).
- 13) Fring C. S., Dunn R., *Am. J. Clin. Pathol.*, **4**, 53-89 (1970).
- 14) Janbaz K. H., Gilani A. H., *Food Chem. Toxicol.*, **37**, 603-607 (1999).
- 15) Brattin W. J., Glende E. A., Recknagel R. O., *J. Free Radic. Bio. Med.*, **1**, 27-38 (1985).
- 16) Mitra S. K., Venkataranganna M. V., Sundaram R., Gopumadhavan S., *J. Ethnopharmacol.*, **63**, 181-186 (1998).
- 17) Seakins A., Robinson D. S., *Biochemistry.*, **86**, 401-407 (1963).
- 18) Dubey G. P., Agrawal A., Dixit S. P., *Antiseptic*, **91**, 205-208 (1994).