Medicinal and Aromatic Plant Science and Biotechnology ©2011 Global Science Books

Hepatoprotective Effect of *Bauhinia variegata* (Linn.) Whole Stem against Carbon Tetrachloride-Induced Hepatopathy in Rats

Saumya Ranjan Pani[1] • Sabuj Sahoo[1*] • Prasana Kumar Panda[1] • Satyaranjan Mishra[1]

[1] University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar –751 004, Orissa, India
[2] K.V. Virani Institute of Pharmacy & Research Centre, Badhada, Savarkundla-364 515, Gujarat, India

Corresponding author: * *sabujbiotech@rediffmail.com*

ABSTRACT

The hepatoprotective activity of the ethanolic extract of *Bauhinia variegata* (Linn.) whole stem (BV) against carbon tetrachloride (CCl_4) induced hepatic failure was investigated by *in vitro* and *in vivo* methods. The *in vitro* method resulted in a significant ($p<0.001$) increase in enzyme levels (AST, 36.21 ± 2.78 IU/l; ALT, 23.14 ± 1.91 IU/l and ALP, 37.3 ± 2.35 IU/l) following CCl_4 treatment of liver explant cultures (toxic control) as compared to the normal control (AST, 11.55 ± 2.27 IU/l; ALT, 2.32 ± 1.17 IU/l and ALP, 12.1 ± 1.5 IU/l). BV extract at a dose of 3.3 mg/ml significantly lowered the levels of the enzymes, AST (20.99 ± 2.12 IU/l; $p<0.01$) and ALP (21.79 ± 1.82 IU/l; $p<0.001$). Acute hepatotoxicity (*in vivo*) was induced by intra-peritoneal (i.p.) injection of CCl_4 (CCl_4 + olive oil in a 1 : 1 ratio; 2 ml/kg). Administration of BV extract at a dose of 200 and 400 mg/kg of body weight to CCl_4-treated rats for 7 days attenuated the marker enzyme level of liver, in a dose-dependant manner. BV extract at 400 mg/kg decreased the level of marker enzymes (AST, 272.77 ± 24.08 IU/l; ALT, 189.15 ± 7.16 IU/l and ALP, 97.15 ± 6.54 IU/l) significantly ($p<0.001$) with a significant ($p<0.001$) increase in body weight (6.16 ± 1.01 g) as compared to the toxic control group. The ethanolic extract of BV (400 mg/kg) exhibited significant and comparable hepatoprotective potential as that of the standard polyherbal drug Liv-52. The statistically significant results obtained in this study thus suggests a protective function of *B. variegata* whole stem against CCl_4-induced hepatopathy both *in vitro* and *in vivo*.

Keywords: hepatotoxicity, hepatotoxin, kanchnara, orchid tree, marker enzymes, oxidative stress, lipid peroxidation
Abbreviations: ALP, Alkaline phosphatase; **ALT**, Alanine aminotransferase; **AST**, Asparate transaminase; **BV**, *Bauhinia variegata*; **CMC**, carboxy methyl cellulose; **DMEM**, Dulbeccos modified Egale's medium; **i.p.**, intra peritoneal; **OECD**, Organization for Economic Co-operation and Development

INTRODUCTION

The mammalian liver is not only critical for the overall health and metabolic activity of an individual but also functions as an important site for cellular detoxification. Thus, any dysfunction of the liver (hepatopathy) can potentially lead to multi-organ failure and death eventually. Hepatopathy, of varied etiology is encountered globally irrespective of age, ethnic, racial backgrounds or environmental and geographical diversity. The reasons of hepatopathy incidence and associated fatalities are multifaceted which range from chemicals, drugs or substance induced hepatic anomaly to various metabolic and physiological disturbances causing hepatotoxicity and leading to liver damage. This places hapatopathy as one of the leading cause of death across the world (Friedman *et al*. 2003; Bernal *et al*. 2010).

Carbon tetrachloride (CCl4) is a widely used industrial chemical and is also a potent hepatotoxin. It induces hepatotoxicity due to free radical production thereby inducing oxidative stress and lipid peroxidation in liver tissue and consequently, causing irreversible liver damage by necrosis (Ram *et al*. 1999; Weber *et al*. 2003; Maddrey 2005).

Bauhinia variegata Linn. (BV; family Caesalpiniaceae) is a deciduous plant widely distributed throughout tropical and subtropical regions of the world including India and China. It is commonly known as 'orchid-tree', 'mountain eboney' and its local Indian names are 'kanchnara' and 'kanchana' (Chopra *et al*. 1956). This plant species has been used as a medicinal plant and cattle feed by the tribes of India (Nadkarni 1954; Kirtikar *et al*. 1999) and has also been used in various indigenous systems of medicine like Ayurveda and Unani (The Ayurvedic Pharmacopoeia of India 1990). Different parts of this plant are used traditionally for various aliments: as a liver tonic, antibacterial and to suppress the edema arising because of kidney failure (Mali *et al*. 2007). This plant has undergone vast pharmacological screening in the past decade and has been observed to have beneficial effects against many ailments in various experimental animal models. The bark extracts of BV have been shown to have concentration-dependent antibacterial activity with more sensitivity to Gram-negative than Gram-positive bacteria (Parekh *et al*. 2006). The chemopreventive and cytotoxic effects of BV were evaluated in N-nitrosodiethylamine (DEN)-induced experimental liver tumor in rats and human cancer cell lines and was found to be cytotoxic against human epithelial larynx cancer (HEp2) and human breast cancer (HBL-100) cells (Rajkapoor *et al*. 2006). The ethanolic extract of BV at a dose of 250 mg/kg for 15 days has been demonstrated to have a significant inhibitory action on Complete Freund's adjuvant-induced arthritis in Wistar rats (Rajkapoor *et al*. 2007). More recently, the ethanolic and aqueous extracts from stem, bark and roots of BV have been demonstrated to possess antioxidant and antihyperlipidemic property both *in vitro* and *in vivo*, respectively (Rajani *et al*. 2009). Taken together the evidence mentioned above strongly demonstrates a significant medicinal value of BV. However, the protective effect of BV whole stem against CCl4-induced hepatotoxicity in rats has not been substantially explored. Thus, the present study was aimed to evaluate the hepatoprotective potential of the ethanolic extract of BV whole stem in a conventional model (*in vivo* and *in vitro*) of CCl4-induced hepatopathy.

Received: 12 February, 2010. Accepted: 10 November, 2010.

Original Research Paper

MATERIALS AND METHODS

The whole stem of BV was collected from young matured plant from Bharatpur Forest Range, Bhubaneswar, Orissa, India during Nov-Dec and identified by Dr. B. K. Jaysingh, Gopabandhu Ayurvedic College, Puri, Orissa, India. A plant specimen (vide Voucher No: 081) was deposited in the herbarium of the University Department of Pharmaceutical Sciences, Utkal University, Bhubaneswar, Orissa, India. After authentication, fresh plant materials were collected in bulk, washed under running tap water to remove adherents, shade dried and pulverized in a mechanical grinder to produce coarse powdered plant material. The coarse BV whole stem powder was used for solvent extraction.

All animals used in the study were healthy male albino rats of Wistar strain weighing between 150-200 g (purchased from Ghosh Enterprises, Kolkata, India). The animals were maintained under standard laboratory conditions (12 ± 1 hr, day-night schedule; temperature maintained between 11-20 ± 2°C; housed in large spacious hygienic cages with access to food and water *ad libitum*) and were acclimatized for one week prior to the experiments. The experimental protocol was approved by the Institutional Animal Ethics Committee (Vide Registration No.: 990/c/06/CPCSEA).

Chemicals used in the study were of analytical grade and were procured from Merck Specialties Pvt., Ltd., Mumbai, India. Dulbeccos Modified Eagle's Medium (DMEM) was procured form Sigma, USA. Liv-52 (standard polyherbal drug-Liv-52 syrup; Himalya Dug Company, India), Silymarin (Silybon suspension; Micro Labs Pvt. Ltd., India) and Phenobarbitone sodium (Neon Labs., India) were purchased from local chemist. All biochemical assay kits were purchased from Ecoline (Merck).

Extraction of plant material and preparation of test dose

About 200 g of coarse BV whole stem powder was taken in a Soxhlet apparatus and extracted successively (Suftness 1979) with selected solvents (petroleum ether → chloroform → ethanol). The extraction for each solvent was carried out for approximately 18-20 hr. Following the ethanol extraction step, the resulting fraction was further evaporated by slow heat treatment to obtain the final ethanolic extract of BV whole stem. A total of 1.4 kg of pulverized stem was used to produce the required amount of test extract.

For *in vivo* administration, calculated amount of dried ethanolic extract of BV was suspended in normal saline solution (normal saline with sodium-CMC; 0.5% w/v) to produce the test doses of 200 and 400 mg/kg/ml respectively. The dose limit was selected on the basis of previously performed oral acute toxicity studies in mice, in accordance with the OECD guidelines (OECD 2000).

Hepatoprotective activity study

1. In vitro hepatoprotective activity

For *in vitro* studies, rat liver was extracted and liver explant culture was obtained by growing cells in DMEM supplemented with 5% new born calf serum and were maintained for 48 hr prior to the experiments as per previously reported protocol (Freshney 2005). A total of 6 independent explant culture sets were taken for the study with each set comprising duplicate plates. The different experimental conditions for all 6 sets of liver explants are as follows, Set 1 (normal control): Pure and unexploited explant culture, Set 2 (toxic control): where CCl_4 (83.3 µl/ml) was added to each plate, Set 3 (standard): where CCl_4 (83.3 µl/ml) + silymarin (0.5 ml; 1.9 mg/ml) (Rao *et al.* 2010) was added to each plate and Sets 4, 5 and 6 (test extract treated explant culture): where BV extact was added to each culture sets in an increasing order of concentration (at 0.825, 1.65 and 3.3 mg/ml, respectively) along with CCl_4 (83.3 µl/ml). The plates with or without the required treatments were then incubated for an additional 2 hr period. Following incubation, the explant culture supernatant samples from all experimental sets were collected and were assayed to estimate the level of various enzymes namely, asparate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP).

2. In vivo hepatoprotective activity

For *in vivo* studies, a total of 30 male Wistar rats were divided randomly into 5 groups, with each group comprising of 6 animals. Group I (normal control) received oral dose of 0.5% Sodium CMC (1 ml each) for 7 days. Group II (toxic control) received single dose of CCl_4 (CCl_4 + olive oil in a 1:1 ratio; 2 ml/kg; i.p.) on day 1 and day 7 of the experiment (Ray *et al*. 2006). Group III (standard) received polyherbal drug 'Liv-52' (5 ml/kg; *p.o.*) (Sandhir *et al.* 1999) with single i.p. dose of CCl_4 on 1st and 7th day, Group IV and V (BV extract treated groups) received BV extract 200 and 400 mg/kg of body weight once a day for 7 days together with a single i.p. dose of CCl_4 on day 1 and day 7, as mentioned above.

Following completion of experimental period, on day 8, rats were anesthetized, using phenobarbitone sodium (40 mg/kg; i.p.) and the blood samples along with liver tissues were collected from each test group. Blood samples were collected from the test animals under anesthesia by direct cardiac puncture at ventricular site before sacrifice and serum parameters of liver function including: AST, ALT, ALP, albumin and total protein were estimated as per reported procedures (Doumas *et al.* 1971; Tietz 1999; Cheesborough 2003). The biochemical estimations were done in a Biochemical-semi-auto analyzer (EBRA-Chem-5 Plus. V2, West-Germany) by standard procedures using commercial kits.

Livers from test animals were removed before sacrifice and the tissue samples were fixed using formosal solution (formaldehyde in normal saline; 10% v/v), embedded with paraffin wax followed by preparation of tissue sections using a microtome for histopathological analysis (Suzuki *et al.* 1990; Sood 2002).

Statistical analysis

Results obtained from both *in vitro* and *in vivo* hepatoprotective studies were tabulated as Mean values ± SEM (standard error of mean). Statistical difference and significance of data were assessed by analysis of variance (one-way ANOVA) followed by comparison between different groups using 'Tukey-Kramer' multiple comparison test. The significance level was set at $p<0.05$. The toxic control group was compared with the normal control group and all other treatment groups were compared with the toxic control group in both (*in vitro* and *in vivo*) methods.

RESULTS AND DISCUSSION

The *in vitro* hepatoprotective activity of BV against CCl_4 was analyzed on rat liver explant cultures grown for 48 hr *in vitro*. To evaluate injury to the hepatic cells, the supernatant collected from the culture sets were processed to estimate the level of marker enzymes- AST, ALT and ALP. As shown in the **Table 1**, the toxic control set (CCl_4-treated explant cultures) resulted in a significant ($p<0.001$) increase in AST (36.21 ± 2.78 IU/l), ALT (23.14 ± 1.91 IU/l) and ALP (37.3 ± 2.35 IU/l) level as compared to normal control set (AST, 11.55 ± 2.27 IU/l; ALT, 2.32 ± 1.17 IU/l and ALP, 12.1 ± 1.5 IU/l). Interestingly, in BV extract treated sets a dose-dependant decrease in the levels of all the estimated enzymes was observed. Treatment of the BV extract at a higher concentration (3.3 mg/ml) significantly lowered the AST (20.99 ± 2.12 IU/l; $p<0.01$) and ALP (21.79 ± 1.82 IU/l; $p<0.001$) level as compared to toxic control set. However, no significant change was observed in ALT level (16.94 ± 1.17 IU/l; $p>0.05$) on comparison with toxic set. The standard set (explant culture incubated with CCl_4 + silymarin) showed an enhanced hepatoprotective efficacy as AST (15.08 ± 1.83 IU/l), ALT (10.02 ± 1.06 IU/l) and ALP (14.85 ± 2.18 IU/l) levels were decreased significantly ($p<0.001$) as compared to CCl_4 treated explants culture set.

The results obtained from our *in vivo* hepatoprotective studies are outlined in **Table 2**. Analogous to the findings from our *in vitro* studies, we found a dose-dependant hepatoprotective effect of BV extract in whole animals. Change in body weight and serum parameters of liver function including AST, ALT, ALP, albumin and total protein were analyzed on 8th day of experiment. As compared to the controls, the CCl_4 administered animals (toxic control

Table 1 Parameters studied for *in vitro* hepatoprotective activity of *Bauhinia variegata* whole stem extract on rat liver explant cultures.

Group	AST (U/l)	ALT (U/l)	ALP (U/l)
Set 1 [control]	11.55 ± 2.27	2.32 ± 1.17	12.1 ± 1.5
Set 2 [CCl_4 (83.3 µl/ml)]	36.21 ± 2.78 c	23.14 ± 1.91 c	37.3 ± 2.35 c
Set 3 [CCl_4 (83.3 µl/ml) + Silymarin (0.5 ml; 1.9 mg/ml)]	15.08 ± 1.83 c	10.02 ± 1.06 c	14.85 ± 2.18 c
Set 4 [CCl_4 (83.3 µl/ml) + BV extract (0.825 mg/ml)]	27.12 ± 2.97	20.09 ± 1.91	28.72 ± 1.72
Set 5 [CCl_4 (83.3 µl/ml) + BV extract (1.65 mg/ml)]	25.19 ± 2.17	17.92 ± 1.94	28.21 ± 1.97
Set 6 [CCl_4 (83.3 µl/ml) + BV extract (3.3 mg/ml)]	20.99 ± 2.12 b	16.94 ± 1.17	21.79 ± 1.82 c

Values are given in mean ± SEM.
a - $p<0.05$, b - $p<0.01$, c - $p<0.001$. For n=6. Toxic control group was compared with Normal control group and all the other groups were compared with toxic control group.
AST- Asparate transaminase, ALT- Alanine aminotransferase, ALP- Alkaline phosphatase, BV- Ethanolic extract of *Bauhinia variegata* whole stem

Table 2 Parameters studied for *in vivo* hepatoprotective activity of *Bauhinia variegata* whole stem extract on rats (8th day).

Group	Change in body weight (g)	Albumin (g/dl)	Total protein (g/dl)	AST (IU/l)	ALT (IU/l)	ALP (IU/l)
Group I (Normal control)	10.66 ± 1.28	2.83 ± 0.17	5.70 ± 0.35	75.04 ± 7.23	49.21 ± 4.90	80.30 ± 3.58
Group II (Toxic control; CCl_4 toxicated)	-12.83 ± 1.13 c	1.40 ± 0.10 c	3.11 ± 0.16 c	840.37 ± 28.26 c	746.75 ± 18.94 c	133.65 ± 3.47 c
Group III (Standard; Liv-52 treated)	6.83 ± 0.70 c	2.71 ± 0.27 c	5.09 ± 0.20 c	246.23 ± 16.93 c	186.38 ± 14.60 c	86.49 ± 7.17 c
Group IV (BV, 200 mg/kg treated)	3.33 ± 1.90 c	2.55 ± 0.16 b	4.70 ± 0.36 b	393.08 ± 12.29 c	241.07 ± 11.73 c	112.58 ± 6.82
Group V (BV, 400 mg/kg treated)	6.16 ± 1.01 c	2.61 ± 0.18 c	4.96 ± 0.31 b	272.77 ± 24.08 c	189.15 ± 7.16 c	97.15 ± 6.54 b

Values are given in mean ± SEM
.a - $p<0.05$, b - $p<0.01$, c - $p<0.001$. For n=6. Toxic control group was compared with Normal control group and all the other groups were compared with toxic control group.
AST- Asparate transaminase, ALT- Alanine aminotransferase, ALP- Alkaline phosphatase, BV- Ethanolic extract of *Bauhinia variegata* whole stem.

group) revealed prominent hepatotoxicity as shown by a significant ($p<0.001$) elevation in marker enzyme levels (AST, 840.37 ± 28.26 IU/l; ALT, 746.75 ± 18.94 IU/l and ALP, 133.65 ± 3.47 IU/l). Additionally, a significant ($p<0.001$) decrease in serum total protein (3.11 ± 0.16 g/dl), albumin (1.40 ± 0.10 g/dl) and final body weight (-12.83 ± 1.13 g) was also observed in toxic control group when compared with the normal control. Standard polyherbal drug (Liv-52) treated rat group showed a significant ($p<0.001$) decrease in serum levels of AST (246.23 ± 16.93 IU/l), ALT (186.38 ± 14.60 IU/l) and ALP (86.49 ± 7.17 IU/l) with significant ($p<0.001$) elevation in serum albumin (2.71 ± 0.27 g/dl), total protein (5.09 ± 0.20 g/dl) and body weight (6.83 ± 0.70 g) when compared with the toxic control group.

Group IV (BV extract 200 mg/kg treated rats) exhibited elevation in body weight (3.33 ± 1.90 g; $p<0.001$). The levels of AST (393.08 ± 12.29 IU/l) and ALT (241.07 ± 11.73 IU/l) were decreased significantly ($p<0.001$). There was a significant ($p<0.01$) increase in serum albumin (2.55 ± 0.16 g/dl) and total protein (4.70 ± 0.36 g/dl) level as compared to the toxic control group. However no significant ($p>0.05$) change was observed in serum level of ALP (112.58 ± 6.82 IU/l).

Group V (BV extract 400 mg/kg treated rats) showed significant ($p<0.001$) elevation in body weight (6.16 ± 1.01 g) associated with a significant ($p<0.001$) decrease in serum levels of AST (272.77 ± 24.08 IU/l) and ALT (189.15 ± 7.16 IU/l) as compared to the toxic control group. Serum total protein (4.96 ± 0.31 g/d; $p<0.01$) and albumin (2.61 ± 0.18 g/dl; $p<0.001$) level was elevated with a significant ($p<0.01$) decrease in the level of ALP (97.15 ± 6.54 IU/l) as compared to the toxic group.

We further performed histological studies to investigate the protective effect of BV extract on liver tissue. Transverse section (TS) of liver tissue samples of different experimental groups are illustrated in the **Fig. 1A-D**. **Fig. 1A** shows the TS of normal control rat with a normal hepato-cellular configuration. In contrast, in **Fig. 1B**, TS of toxic control rat exhibited hepato-cellular injury as evidenced by presence of necrotic patches, inflammatory cells and disturbed hepato-cellular arrangement. The TS of the standard group (**Fig. 1C**) and BV extract (400 mg/kg)-treated group (**Fig. 1D**) showed improved hepato-cellular architecture with near absence of necrotic lesions in contrast to the toxic control group.

Taken together the findings presented here substantially suggest that the BV extract treated rats (*in vivo*) and liver explant cultures (*in vitro*) were significantly protected from CCl_4-induced hepatic injury. Interestingly, the BV extracts exerted a dose-dependant hepatoprotective efficiency both

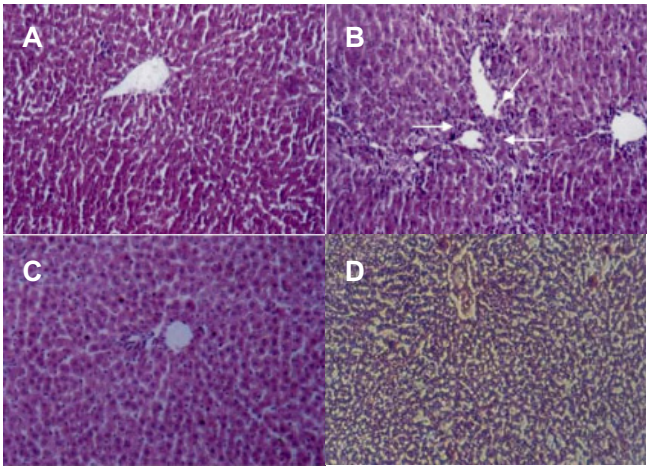

Fig. 1 Transverse sections of liver. (**A**) Group I (Normal control) section shows central vein surrounded by hepatic cord of cells. (**B**) Group II (Toxic control; CCl_4 treated) section shows patches of liver cell necrosis with inflammatory collections around the central vein. (**C**) Group III (Standard; Liv-52 + CCl_4 treated) induction almost near normal. (**D**) Group IV (BV extract, 400 mg.kg + CCl_4)

in vivo and *in vitro*. The BV extract treatments rendered resistance to the hepatic cells against CCl_4 as evident from the improved metabolic functions (at the level of biochemical marker enzymes) and hepato-cellular architecture (at the level of tissue histology) as compared to the toxic group.. Moreover, the results obtained in our treatment groups are comparable with the previously reported hepatoprotective effects of other plant species like *Cassia angustifolia* (Ilavarasan *et al.* 2001), *Bacopa monnieri* Linn. (Ghosh *et al.* 2007), *Pterocarpus santalinus* (Manjunath 2006), *Eucalyptus maculata* (Abdel-Fattah *et al.* 2005) and a poly-herbal preparation, Pricoliv (Dwivedi *et al.* 1990). It is well documented that the BV whole stem contains a variety of active components such as, octacosanol, stigmasterol (Prakash *et al.* 1976), 5, 7-dihydroxy flavonone-4-*O*-α-L rhanmopyranosyl-β-D-glucopyranoside – a flavonone glycoside (Gupta *et al*. 1979), β-sitosterol, lupeol, kaempferol-3-glucoside – a flavone glycoside (Gupta *et al*. 1984) and many other alkaloids, flavonoids and terpenoids (Duret *et al*. 1977; Gupta *et al.* 1980). Thus, we suggest that the hepatoprotective potential of BV whole stem, is because of the active phytochemicals present in it. It was mentioned earlier that CCl_4 is a potent hepato-toxin which induces lipid peroxidation and consequently promotes liver damage or

hepatopathy *in vivo*. Moreover, it has been evident from numerous studies on screening and evaluation of pharmacological effects of phytochemicals that, several phytochemicals such as flavonols, flavonoids, triterpenoids, sterols and alkaloids have the ability to inhibit lipid peroxidation induced by CCl_4 (Baek *et al.* 1996; Mehta *et al.* 1999). Thus, we presume that the active phytochemicals present in BV whole stem (either single or complex molecular components all together) would be responsible for the hepatoprotective activity, exhibited by BV whole stem in our experimental setup. Thus, identification of the precise phytochemicals present in BV whole stem that have the hepatoprotective property will be necessary to advance our understanding in this order.

CONCLUSION

Present study was undertaken to investigate the hepatoprotective potential of *Bauhinia variegata* Linn. (whole stem) against chemical (CCl_4) induced hepatopathy. The result obtained from this study suggested the hepatoprotective activity of BV whole stem against CCl_4-induced hepatotoxicity. The hepatoprotective potential of ethanolic extract of BV whole stem was a dose-dependant effect. Thus, in conclusion our study puts forward the *Bauhinia variegata* Linn. plant as a promising hepatoprotective plant species which, in the future will require a more elaborate research to understand and identify the precise molecular mechanism(s) involved in its hepatoprotective activity.

REFERENCES

Abdel-Fattah M, Abeer G, El-Hammamy MM, Abdel-Sattar E (2005) Antioxidant and hepatoprotective effects of *Eucalyputs maculata*. *Medical Science Monitor* **11**, 426-431

Baek NL, Kim YS, Kyung JS, Park KH (1996) Isolation of anti-hepatotoxic agent from the roots of Astragalus membranaceous. *Korean Journal of Pharmacognosy* **27**, 111-116

Bernal W, Auzinger G, Dhawan A, Wendon J (2010) Acute liver failure. *Lancet* **376**, 190-201

Cheesbrough M (2003) *District Laboratory Practice in Tropical Countries* (Vol 1), Cambridge University Press, United Kingdom, pp 310 -395

Chopra RN, Nayer SL, Chopra IC (1956) *Glossary of Indian Medicinal Plants*, Council of Industrial and Scientific Research, India, 35 pp

Doumas BT, Watson WA, Biggs HG (1971) Albumin standards and the measurement of serum albumin with bromocresol green. *Clinica et Chimica Acta* **31**, 87-96

Duret S, Paris RR (1977) The flavonoids of several species of *Bauhinia*. *Plant Medicinal Phytotherapy* **11**, 213-215

Dwivedi Y, Rastogi R, Chander R, Sharma SK, Kapoor NK, Garg NK (1990) Hepatoprotective activity of Picroliv against carbontetrachloride induced liver damage in rats. *Indian Journal of Medical Research* **92**, 185-200

Freshney RI (2005) *Culture of Animal Cells. A Manual of Basic Techniques* (5th Edn), John Wiley and Sons Inc. Publications, New York, USA, 182 pp

Friedman SE, Grendell JH, McQuaid KR (2003) *Current Diagnosis and Treatment in Gastroenterology*, Lang Medical Books, McGraw-Hill Publications, New York, USA, pp 664-679

Ghosh T, Maity TK, Das M, Boss A, Dash DK (2007) *In vitro* anti-oxidant and hepatoprotective activity of ethanolic extract of *Bacopa monnieri* Linn. aerial parts. *Iranian Journal of Pharmacology and Therapeutics* **6**, 77-85

Gupta AK, Vidyapati TJ, Chauhan JS (1979) 5,7-dihydroxyflavonone-4-*O*-α-L-rhanmopyranosyl-β-D–glucopyranoside from stem of *Bauhinia variegata*. *Indian Journal of Chemistry* **18 (B)**, 85-86

Gupta AK, Vidyapati TJ, Chauhan JS (1980) Chemical examination of the stem of *Bauhinia variegata*. *Planta Medica* **38**, 174-176

Gupta AK, Chauhan JS (1984) Constituents from the stem of *Bauhinia variegata*. *National Academy Science Letters* **7**, 174-176

Ilavarasan R, Mohideen S, Vijayalakshmi M, Manonmani G (2001) Hepatoprotective effect of *Cassia angustifolia* Vahl. *Indian Journal of Pharmaceutical Sciences* **63**, 504-507

Kirtikar KR, Basu BD (1999) *Indian Medicinal Plants*, International Book Distributor, Dehradun, India, pp 892-901

Maddrey WC (2005) Drug-induced hepatotoxicity. *Journal of Clinical Gastroenterology* **39**, 83-89

Mali RG, Mahajan SG, Mehta AA (2007) Rakta Kanchan (*Bauhinia variegata*): Chemistry, traditional and medicinal uses – a review. *Pharmacognosy Reviews* **1**, 314-319

Manjunath BK (2006) Hepatoprotective activity of *Pterocarpus santalinus* L.F., an endangered medicinal plant. *Indian Journal of Pharmacology* **38**, 25-28

Mehta RS, Shankar MB, Geetha M, Saluja AK (1999) Hepatoprotective activity of *Trianthema portulacastrum*. *Indian Drugs* **36**, 241-244

Nadkarni AK (1954) *Indian Materia Medica*, Popular Prakashan, India, pp 184-190

OECD (2000) *Acute Oral Toxicity - Acute Oral Toxic Class Method, Guideline 423*, adopted 23.03.1996. In: 11th Addendum to the OECD guidelines for the Testing of Chemicals. Organization for Economic Co-Operation and Development, Paris

Parekh J, Karathia NN, Chanda S (2006) Evaluation of antibacterial activity and phytochemical analysis of *Bauhinia variegata* L. bark. *African Journal of Biomedical Research* **9**, 53-56

Prakash A, Khosa RL (1976) Chemical studies on *Bauhinia variegata*. *Current Science* **45**, 705-706

Rajkapoor B, Jayakar B, Murgesh N, Sakthisekaran D (2006) Chemoprevention and Cytotoxic effect of *Bauhinia variegata* against *N*-nitrosodiethylamine-induced liver tumors and human cancer cell lines. *Journal of Ethnopharmacology* **104**, 407-409

Rajkapoor B, Ravichandran V, Gobinath M, Anbu J, Harikrishnan N, Sumithra M, Sankari M, Venugopal R, Sakthisekaran D (2007) Effect of *Bauhinia variegata* on complete Freund's adjuvant induced arthritis in rats. *Journal of Pharmacology and Toxicology* **2**, 465-472

Rajani GP, Ashok P (2009) *In vitro* antioxidant and antihyperlipidemic activities of *Bauhinia variegata* Linn. *Indian Journal of Pharmacology* **41**, 227-232

Ram VJ, Goel A (1999) Past and present scenario of hepatoprotectants. *Current Medicinal Chemistry* **6**, 217-254

Rao CM, Raj PV, Nitesh K, Chandrashekhar HR, Rao JV, Udupa N (2001) Effect of lecithin and silymarin on D-Galactosamine induced toxicity in isolated hepatocytes and rats. *Indian journal of Clinical Biochemistry* **25**, 169-174

Ray D, Sharatchandra Kh, Thokchom IS (2006) Antipyretic, antidiarrhoeal, hypoglycemic and hepatoprotective activities of ethyl acetate extract of *Acacia catechu* Wild. in albino rats. *Indian Journal of Pharmacology* **38**, 408-413

Sandhir R, Gill KD (1999) Hepatoprotective effects of Liv.52 on ethanol-induced liver damage in rats. *Indian Journal of Experimental Biology* **37**, 762-766

Sood R (2002) *Medical Laboratory Technology: Methods and Interpretations* (4th Edn), Jayepee Brothers Publication Pvt. Ltd., India, 224 pp

Suftness M, Douras J (1979) *Drugs of Plant Origin in Methods in Cancer Research*, Academic Press, New York, 548 pp

Suzuki CAM, Cherian MG (1990) Interaction of *cis*-diaminedichloroplatinum with metallothionein and glutathione in rat liver and kidney. *Toxicology* **64**, 113-127

The Ayurvedic Pharmacopoeia of India (1990) Ministry of Health and Family Welfare, Government of India, 10 pp

Tietz NW, Burtis CA, Ashwood ER (1999) *Tietz Text Book of Clinical Chemistry* (3rd Edn), W.B Saunders Co., Philadelphia, USA, pp 617-721

Weber LW, Boll M, Stampfl A (2003) Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. *Critical Reviews in Toxicology* **33**, 105-136