OPEM
www.opem.org

Oriental Pharmacy and Experimental Medicine 2007 **7(3)**, 297-303

KHUP

Effect of various fractions of *Bacopa monnieri* Linn. aerial parts on ethanol-induced hepatotoxicity in rats

Tirtha Ghosh[1,*], Tapan Kumar Maity[2], Deepak Kumar Dash[2] and Anindya Bose[1]

[1]Institute of Pharmacy and Technology, Salipur, Cuttack, Orissa -754202, India; [2]Department of Pharmaceutical Technology, Jadavpur University, Kolkata-700032 India

SUMMARY

The ethyl acetate fraction (EAF) and n-butanol fraction (NBF) of ethanolic extract of *Bacopa monnieri* aerial parts were screened for hepatoprotective activity and *in vivo* antioxidant activity on ethanol-induced hepatotoxic rats. Ethyl acetate fraction was found to be more potent even though both the fractions were endowed with significant hepatoprotective activity. EAF and NBF were investigated for hepatoprotective activity in albino rats at 300 mg/kg, p.o. dose and compared with standard drug Silymarin (25 mg/kg, p.o.). Results show that both the fractions were effective in blunting ethanol-induced enhanced activities of serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, alkaline phosphatase, level of serum bilirubin (both total and direct), liver weight loss and was also effective in reducing ethanol-induced lipid peroxidation both *in vitro* and *in vivo*. Furthermore, the fractions could also enhance ethanol-induced suppressed activities of superoxide dismutase, catalase and decreased level of reduced glutathione. Results of hepatocellular damage caused by ethanol and its recovery by EAF and NBF, suggest that they might be considered as a potential source of natural hepatoprotective agents, which could be related to the free radical scavenging properties of saponins present in high concentration in the fractions.

Key words: *Bacopa monnieri*; Hepatoprotective activity; Antioxidants; Saponins

INTRODUCTION

Alcohol dependency is a major health and socio-economic problem throughout the world (Sandhir *et al.*, 1999; Rezvani *et al.*, 2003). It has been observed that almost all ingested alcohol is metabolized in the liver and excessive alcohol use can lead to acute and chronic liver disease. It has further been observed that most of the consumed alcohol is eventually broken down by the liver and the products generated and accumulated during alcohol metabolism (e.g. acetaldehyde) are more toxic than alcohol itself. In addition, a group of metabolic products called free radicals can damage liver cells and promote inflammation, impairing vital functions such as energy production. The body's natural defenses against free radicals (e.g. antioxidants) are inhibited by alcohol consumption, leading to increased liver damage (Kurose *et al.*, 1996). Despite great progress made in the field in the past two decades, development of suitable medications for the treatment of alcohol dependency or alcohol-induced health injury remains a challenging goal for alcohol research.

Bacopa monnieri Linn. (Scrophulariaceae) is a creeping, glabrous, succulent herb, rooting at nodes, distributed throughout India in all plain districts, ascending to an altitude of 1,320 m (Anonymous, 1998). The plant is reported to contain tetracyclic triterpenoid saponins, bacopasides A, B, C, hersaponin, alkaloids viz.

*Correspondence: Tirtha Ghosh, Institute of Pharmacy and Technology, Salipur, Cuttack, Orissa -754202, India. E-mail: tghosh75@yahoo.co.in

herpestine and brahmin and flavonoids (Kiritikar and Basu, 1994; Hou *et al.*, 2002).

In Ayurveda, the plant has been used in the treatment of insanity, epilepsy and hysteria (Chopra *et al.*, 1986). The other reported activities include sedative, antiepileptic, vasoconstrictor and antiinflammatory respectively (Anonymous, 1998). The ethanolic extract of the aerial parts of the plant has been reported to possess significant anthelmintic activity (Ghosh *et al.*, 2005), and antimicrobial activity (Ghosh *et al.*, 2006a).

Hepatoprotective activity of the ethanolic extract of *Bacopa monnieri* on ethanol-induced hepatotoxic rats has already been reported by the authors (Ghosh *et al.*, 2006b). In the present study the hepatoprotective activity of the ethyl acetate and the n-butanol fractions (both are saponin rich fractions) of the ethanol extract of *Bacopa monnieri* are investigated in a scientific manner.

MATERIALS AND METHODS

Plant material

The plant was identified by the taxonomists of the Botanical Survey of India, Govt. of India, Shibpur, Howrah. After authentication, fresh aerial parts of the young and matured plants were collected in bulk from the rural belt of Salipur, Orissa, India during early summer, washed, shade dried and then milled in to coarse powder by a mechanical grinder. The powdered plant material (400 g) was defatted with petroleum ether (60 - 80°C) and then extracted with 1.5 l of ethanol (95%) in a soxhlet apparatus. The solvent was removed under reduced pressure, which obtained a greenish-black sticky residue (yield: 11.6% w/w with respect to dried plant material). The residue was partitioned successively between ethyl acetate-water system and then between n-butanol-water system. The respective solvents were removed similarly under reduced pressure, which produced ethyl acetate fraction (yield: 48.28% w/w with respect to ethanolic extract) and n-butanol fraction (yield: 24.74% w/w with respect to ethanolic extract). The ethyl acetate and the n-butanol fractions of the ethanolic extract were referred to as EAF and NBF respectively.

Animals

Male Wistar rats weighing 120 ± 5 g were used in the experiment and Swiss albino mice of either sex weighing between 25 - 30 g were used for acute toxicity study. They were maintained in a 12 h light/dark cycle at 25 ± 2°C. They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC).

Diet and treatment

Animals were divided into five groups: Group I (control), Group II (ethanol treated), Group III (ethanol + EAF), Group IV (ethanol + NBF), Group V (ethanol + silymarin). Animals of groups II, III, IV and V were fed with 15% (v/v) ethanol (Luma and Vorne, 1976) at a single dose of 1 ml/100 g body weight per day for 30 days by oral route. Simultaneously, but at different hours of the day, animals of groups III, group IV and group V were fed with 300 mg/kg EAF orally, 300 mg/kg NBF orally and 25 mg/kg silymarin orally for 30 days at a single dose of 1 ml/100 g body wt/day respectively. Animals of group I was administered orally deionized water, 1 ml/100 g body wt/day, as vehicle. Daily records of body weight of all groups of animals were maintained during the whole experimental period.

Acute toxicity study

The test was carried out as suggested by Seth *et al.* (1972). Swiss albino mice of either sex weighing between 25 - 30 g were divided into different groups of six animals in each. The control group received normal saline (2 ml/kg, p.o.). The other groups received 100 - 3,000 mg/kg of the test fractions, respectively. Immediately after dosing, the animals were observed continuously for the first 4 h for any behavioral changes. They were then kept under

observation up to 14 days after drug administration to find out the mortality if any.

Serum analysis

After the treatment period, the animals of all groups were anaesthetized and sacrificed. Blood was drawn from heart and serum was separated for the assay of serum glutamate oxaloacetate transaminase (SGOT) (Rietman and Frankel, 1957), serum glutamate pyruvate transaminase (SGPT) (Rietman and Frankel, 1957), alkaline phosphatase (ALP) (King, 1965) and bilirubin (direct and total) (Malloy and Evelyn, 1937) using analytical kits from Span Diagnostics Ltd., Surat, India.

In vitro antioxidant activity

The inhibitory effect of EAF and NBF on ethanol-induced lipid peroxidation in mice liver homogenate was determined using TBA-MDA adduct according to the modified method of Yuda *et al.* (1999). A mixture containing 0.5 ml of normal liver homogenate, 0.1 ml of Tris- HCl buffer (pH 7.2), 0.1 ml of ethanol and 0.05 ml of various concentrations of test fractions (0.01, 0.1 and 1.0 mg/ml) were incubated for 1 h at 37°C. After incubation 9 ml of distilled water and 2 ml of 0.6% Thiobarbituric acid (TBA) were added to 0.5 ml of incubated solution and shaken vigorously. The mixture was heated for 30 min in a boiling water bath. After cooling, 5 ml of n-butanol was added and the mixture was again shaken vigorously. The n-butanol layer was separated by centrifugation at 1,000 g for 30 min and the absorbance was measured at 532 nm (Wong *et. al,* 1987).

In vivo antioxidant activity

After the treatment period following study, the animals were deprived of food overnight and sacrificed by cervical dislocation. The livers were dissected out, washed in ice-cold saline, patted dry and weighed. A 10% w/v of homogenate was prepared in 0.15 M Tris-HCl buffer and processed for the estimation of lipid peroxidation (LPO) (Fraga *et al.,* 1988). A part of homogenate after precipitating proteins with trichloro acetic acid (TCA) was used for estimation of reduced glutathione (GSH) (Ellman *et al.,* 1959). The rest of the homogenate was centrifuged at 15,000 rpm for 15 min at 4°C. The supernatant thus obtained was used for the estimation of superoxide dismutase (SOD) (Kakkar *et al.,* 1984) and catalase (CAT) activity (Maehly *et al.,* 1954). Protein estimation was done as per the method of Lowry *et al.* (1951).

Statistical analysis

Statistical significance was determined by one way analysis of variance followed by Dunnet's *t*-test ($P < 0.001$) to compare group means.

RESULTS

In acute toxicity study, it was found that the extract induced sedation and temporary postural defect at all tested doses. However, there was no mortality at any of the tested doses till the end of 14 days of observation.

Rats subjected to ethanol only, developed significant ($P < 0.001$) hepatocellular damage as evident from significant increase in serum activities of GOT, GPT, ALP and bilirubin concentration as compared to normal control group, which has been used as reliable markers of hepatotoxicity (Table 1). Oral administration of EAF (300 mg/kg) and NBF (300 mg/kg) exhibited significant reduction ($P < 0.001$) in ethanol-induced increase in levels of GOT, GPT, ALP and bilirubin concentration. Treatment with silymarin also reversed the hepatotoxicity significantly ($P < 0.001$). Liver weight of rats treated with ethanol only decreased significantly ($P < 0.001$), which is prevented by both EAF and NBF that is comparable with silymarin (Table 1). In all the above mentioned parameters studied EAF was found to be more potent than NBF.

Ethanol-induced *in vitro* lipid peroxidation study revealed that EAF and NBF has significant anti lipid peroxidation potential with IC_{50} value being 158.93 μg/ml and 346.45 μg/ml respectively which is

Table 1. Effect of EAF and NBF (300 mg/kg, p.o.) on SGOT, SGPT, ALP, bilirubin and liver weight in ethanol-induced hepatotoxicity in rats

Group	Treatment	SGOT (U/ml)	SGPT (U/ml)	ALP (KA units)	Bilirubin (mg/dl)		Liver weight (g)
					Total	Direct	
I	Control	46.33 ± 0.95	55.33 ± 0.67	78.00 ± 1.79	0.57 ± 0.02	0.08 ± 0.01	10.90 ± 0.45
II	Ethanol treated	135.17 ± 3.11*	117.33 ± 3.98*	159.33 ± 4.53*	6.05 ± 0.33*	0.73 ± 0.04*	8.53 ± 0.25*
III	Ethanol + EAF	53.83 ± 3.55**	56.83 ± 3.79**	108.33 ± 5.10**	0.90 ± 0.03**	0.15 ± 0.03**	10.33 ± 0.42**
IV	Ethanol + NBF	66.50 ± 3.11**	67.17 ± 3.72**	119.17 ± 3.44**	1.15 ± 0.09**	0.28 ± 0.04**	9.33 ± 0.34**
V	Ethanol + Silymarin	53.50 ± 2.95**	62.00 ± 2.92**	91.33 ± 5.06**	0.96 ± 0.06**	0.34 ± 0.04**	10.50 ± 0.38**

Values are mean ± S.E.M. (n = 6). *$P < 0.001$ vs. group I, **$P < 0.001$ vs. group II.

Table 2. Effect of EAF and NBF on ethanol-induced *in vitro* lipid peroxidation

Sample	Concentration (mg/ml)	% inhibition	IC_{50} (mg/ml)
Ethyl acetate fraction (EAF)	50	26.95 ± 1.14	158.93
	100	42.20 ± 2.02	
	200	61.27 ± 1.37	
	300	76.81 ± 1.99	
	400	84.54 ± 3.03	
n-butanol fraction (NBF)	50	12.78 ± 1.01	346.45
	100	21.83 ± 1.35	
	200	36.36 ± 1.10	
	300	41.95 ± 1.66	
	400	56.47 ± 2.87	
a-tocopherol			190.22

Number of observations = 3, Values are mean ± S.E.M.

comparable with the reference drug a-tocopherol (Table 2). IC_{50} value also reveals EAF to be more potent than NBF.

In vivo lipid peroxidation study revealed that ethanol treated group showed significant increase ($P < 0.001$) in malondialdehyde (MDA) level when compared with normal control group. EAF, NBF and silymarin were able to significantly prevent ($P < 0.001$) this rise in MDA level (Fig. 1). The figure shows that EAF is slightly more potent than NBF.

There was a marked decrease in the activities of SOD, CAT and in the level of GSH in ethanol-treated group when compared with normal control group. The activities of SOD, CAT and GSH level were significantly increased ($P < 0.001$) in EAF, NBF and silymarin treated groups (Figs. 2-4). These studies also corroborate the fact that EAF was more potent than NBF.

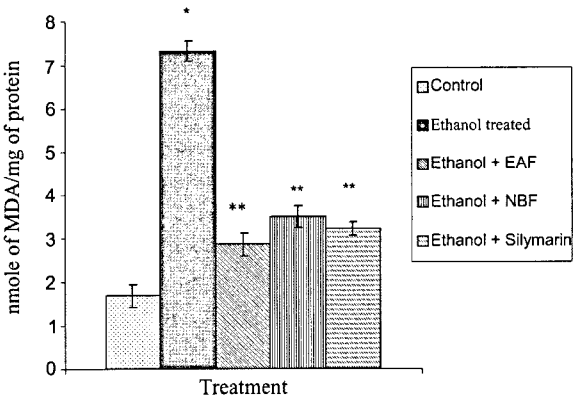

Fig. 1. Effect of EAF and NBF (300 mg/kg, p.o.) on TBARS level in liver of ethanol-induced hepatotoxic rats *in vivo*. Values are mean ± S.E.M. (n = 6). *$P < 0.001$ vs. control group, **$P < 0.001$ vs. ethanol-treated group.

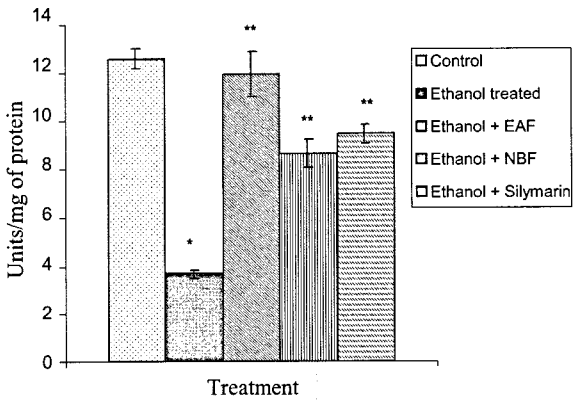

Fig. 2. Effect of EAF and NBF (300 mg/kg, p.o.) on SOD activity in liver of ethanol-induced hepatotoxic rats *in vivo*. Values are Mean ± S.E.M. (n = 6). *$P < 0.001$ vs. control group, **$P < 0.001$ vs. ethanol-treated group.

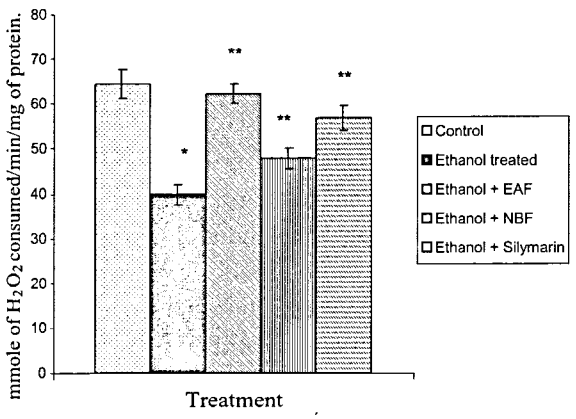

Fig. 3. Effect of EAF and NBF (300 mg/kg, p.o.) on CAT activity in liver of ethanol-induced hepatotoxic rats *in vivo*. Values are mean ± S.E.M. (n = 6). $^{*}P < 0.001$ vs. control group, $^{**}P < 0.001$ vs. ethanol-treated group.

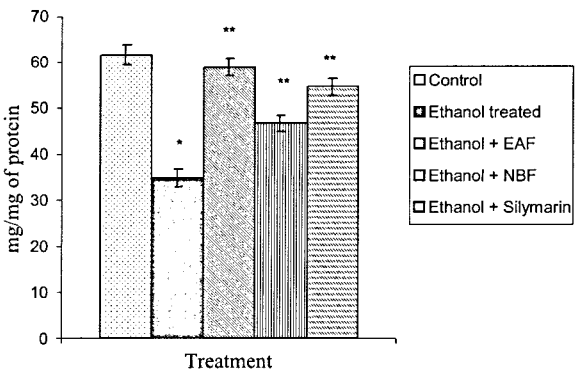

Fig. 4. Effect of EAF and NBF (300 mg/kg, p.o.) on GSH level in liver of ethanol-induced hepatotoxic rats *in vivo*. Values are mean ± S.E.M. (n = 6). $^{*}P < 0.001$ vs. control group, $^{**}P < 0.001$ vs. ethanol-treated group.

DISCUSSION

It has been found that EAF and NBF effectively could prevent ethanol-induced biochemical changes of liver toxicity. The hepatoprotective activity of the ethanolic extract was monitored by estimating serum transaminases, serum alkaline phosphatase and bilirubin, which give a good idea about the functional state of the liver (Rao and Mishra, 1997). The increase in the levels of serum bilirubin reflected the level of jaundice and increase of transaminases and ALP was the clear indications of cellular leakage and loss of functional integrity of cell membrane (Saraswat *et al.*, 1993).

There was a significant decrease in mean liver weight of the animals in ethanol-induced group, which could be blunted significantly by both the fractions. This decrease in liver weight by ethanol-induced hepatotoxicity and its recovery by the fractions, suggests that they possibly have a positive anabolic effect.

Formation of ROS, oxidative stress and hepatocellular injury have been implicated to alcoholic liver disease. It has been documented that Kupffer cells are the major sources of ROS during chronic ethanol consumption, and these are primed and activated for enhanced formation of pro-inflammatory factors (Bautista, 2001). Additionally, alcohol-induced liver injury has been associated with increased amount of lipid peroxidation (Nanji *et al.*, 2003). Indeed, EAF and NBF supplementation in our study was potentially effective in blunting lipid peroxidation, suggesting that they possibly have antioxidant property to reduce ethanol-induced membrane lipid peroxidation and thereby to preserve membrane structure. It may thus be plausible that in our study, loss of membrane structure and integrity because of lipid peroxidation was accompanied with an elevated level of activities of SGOT, SGPT, ALP and bilirubin.

Our study further revealed that chronic exposure to ethanol decreased the activities of the ROS scavenging enzymes, viz. SOD and CAT. This is in line with assumption suggested earlier by (Sandhir and Gill, 1999), that decrease in the activity of antioxidant enzymes SOD, CAT following ethanol exposure may be due to the damaging effects of free radicals, or alternatively could be due to a direct effect of acetaldehyde, formed from oxidation of ethanol, on these enzymes. In our studies, it revealed that both EAF and NBF could restore the activity of both these antioxidant enzymes and possibly could reduce generation of free radicals and hepatocellular damage.

GSH is a naturally occurring antioxidant important

in the antioxidant defence of the body. It has been reported that determination of GSH, can serve as a key to know the amount of antioxidant reserve in the blood and probably in the organism and also, contribute in evaluating the possibilities available for the recuperation of alcoholic patients (Lu *et al.*, 1999; Zentella, 1995). Therefore, the levels of glutathione are of critical importance in liver injury caused by toxic substances such as ethanol. It has been claimed that binding of acetaldehyde, a metabolite of ethanol, with GSH may contribute to reduction in the levels of GSH (Sandhir and Gill, 1999). Our results are in line with this earlier report because we found that after EAF and NBF-supplementation, elevated GSH level in rats with ethanol could be blunted to almost normal level. This ability of the fractions to protect the liver from ethanol-induced damage might be attributed to its ability to restore the activity of antioxidative enzymes as well as GSH level. Thus, results of these studies together with those of earlier ones, suggest that both EAF and NBF has an ability to protect the liver from ethanol-induced damage through its direct antioxidative effect.

In summary, we demonstrate that both the fractions prevent ethanol-induced oxidative stress and hepatic injury. Since these models of hepatic damage in the rat simulate many of the features of human liver pathology, we suggest that natural antioxidants and scavenging agents in these fractions might be effective as plant hepatoprotectors and thus may have some obvious therapeutic implications. Therefore, it seems logical to infer that these fractions, because of their antioxidant property, might be capable of protecting the hepatic tissue from ethanol-induced liver injury. Saponins are natural products, which have been shown to possess antioxidant property (Yoshiki and Okubo, 1995; Yoshiki *et al.*, 1998; Hu *et al.*, 2002). Studies have confirmed that indeed oxidative stress plays an important role in the initiation and progression of alcohol-induced liver disease (ALD) (Davlos and Rolnaldo, 1998; Bautista, 2001; Arteel, 2003). As both EAF and NBF are saponin rich fractions, it might be suspected that the hepatoprotective activity might be due to the presence of saponins in the extract. The activity may be attributed to their protective action on lipid peroxidation and at the same time the enhancing effects on cellular antioxidant defense contributing to the protection against oxidative damage in ethanol-induced hepatotoxicity. As EAF was found to be more potent than NBF, hence further studies regarding the isolation and characterisation of the active principles responsible for hepatoprotective activity from the EAF fraction is currently under progress.

ACKNOWLEDGEMENTS

The authors are thankful to the management of Institute of Pharmacy and Technology, Salipur, India and the authorities of Jadavpur University, Kolkata, India for providing necessary facilities to carry out the research work. The authors are also thankful to the taxonomists of Botanical Survey of India, Shibpur, Howrah, India for proper identification of the plant.

REFERENCES

Anonymous. (1985) Indian Herbal Pharmacopoeia. Vol. I, pp. 30-36, Indian Drug Manufacturer's Association, Mumbai, India.

Arteel GE. (2003) Oxidants and antioxidants in alcohol-induced liver disease. *Gastroenterology* **124**, 778-790.

Bautista AP. (2001) Free radicals, chemokines and cell injury in HIV-I and SIV infections and alcoholic hepatitis. *Free Radic. Biol. Med.* **31**, 1527-1532.

Chopra RN, Nayar SL, Chopra IC. (1986) Glossary of Indian Medicinal plants. pp. 32, National Institute of Science and Communication (CSIR), New Delhi, India.

Davlos M, Rolnaldo N. (1998) Alcoholic hepatitis. *Rev Gastroenterol Peru* **18**, 151-164.

Ellman GL. (1959) Tissue sulphydryl groups. *Arch Biochem Biophys.* **82**, 70-77.

Fraga CG, Leibovitz BE, Toppel AL. (198l) Lipid peroxidation measured as TBARS in tissue characterization and comparison with homogenates

and microsomes. *Free Radic. Biol. Med.* **4**, 155- 161.

Ghosh T, Maity TK, Bose A, Dash GK. (2005) Anthelmintic activity of *Bacopa monnieri. Indian J. Nat. Prod.* **21**, 16-19.

Ghosh T, Maity TK, Bose A, Dash GK. (2006a) A study on antimicrobial activity of *Bacopa monnieri.* Linn. Aerial parts. *J. Nat. Rem.* **6**, 170-173.

Ghosh T, Maity T K, Das M, Bose A, Dash GK. (2006b) Hepatoprotective activity of *Bacopa monnieri* L. against ethanol-induced hepatotoxicity in rat. *Pharmacognosy Magazine* (In Press).

Hou CC, Lin SJ, Cheng JT, Hsu FL. (2002) Bacopaside III, bacopasaponin G, and bacopaside A, B and C from *Bacopa monnieri. J. Nat. Prod.* **65**, 1759-1763.

Hu J, Lee SO, Hendrich S, Murphy PA. (2002) Quantification of the group B soyasaponins by high-performance liquid chromatography. *J. Agric. Food Chem.* **50**, 2587-2594.

Kakkar P, Das B, Viswanathan PN. (1984) A modified spectrophotometric assay of superoxide dismutase. *Indian J. Biochem. Biophys.* **21**, 131-132.

King J. (1965) The hydrolases-acid and alkaline phospatase In: Van, D. (Ed), Practical Clinical Enzymology, pp. 191-208, Nostrand company Ltd., London.

Kiritikar KR, Basu BD. (1994) Indian medicinal plants, Vol I, pp. 1816, Bishen Singh Mahendrapal Singh, Dehradun, India.

Kurose I, Higuchi H, Kato S, Miura S, Ishii H. (1996) Ethanol-induced oxidative stress in the liver. *Alcohol. Clin. Exp. Res.* **20**, 77A-85A.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. (1951) Protein measurement with the folin- phenol reagent. *J. Biol. Chem.* **193**, 265-275.

Lu SC, Huang ZZ, Yang JM, Tsukamoto H. (1999) Effect of ethanol and high-fat feeding on hepatic gamma-glutamylcysteine synthetase subunit expression in the rat. *Hepatology* **30**, 209-214.

Luma P, Vorne M. (1976) Changes in the hepatic microsomal enzyme activity during long-term ethanol feeding in rat. *Acta Pharmacol. Toxicol.* **38**, 260-266.

Maehly AC, Chance B. (1954) Methods of Biochemical Analysis, Vol. I, pp. 357-358, Interscience, New York.

Malloy HT, Evelyn KA. (1937) The determination of bilirubin with the photometric colorimeter. *J. Biol. Chem.* **119**, 481-490.

Nanji AA, Jokelainen K, Tipoe GL Rahemtulla A, Thomas P, Dannenberg AJ, Fisher AE. (2003) Curcumin prevents alcohol-induced liver disease in rats by inhibiting the expression of NFKB- dependent genes. *Am. J. Physiol. Gastrointest. Liver Physiol.* **284**, G321-G327.

Rao SK, Mishra SH. (1997) Anti-inflammatory and hepatoprotective activities of *Sida rhombifolia* Linn. *Indian J. Pharmacol.* **29**, 110-116.

Rezvani AH, Overstreet DH, Perfumi M, Massi M. (2003) Plant derivatives in the treatment of alcohol dependency. *Pharmacol. Biochem. Behav.* **75**, 593-606.

Rietman S, Frankel S. (1957) A colorimetric determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* **28**, 56-63.

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

Saraswat B, Visen PK, Patnaik GK, Dhawan BN. (1993) Anticholeststic effect of Picroliv, active hepatoprotective principle of Picrorhiza kurrooa against CCl_4 induced cholestasis. *Indian J. Exp. Biol.* **31**, 316-318.

Seth UK, Dadkar NK, Kamat UG. Selected Topics in Experimental Pharmacology. (1972) 1st ed., pp. 126, Kothari Book Depot, Bombay, India.

Wong SH, Knight JA, Hopfer SM. (1987) Lipoperoxide in plasma as measured by lipid chromatographic separation of malondialdehyde- thiobarbituric acid adduct. *Clin. Chem.* **33**, 214-220.

Yoshiki K, Kudou S, Okubo K. (1998) Relationship between chemical structures and biological activities of triterpinoid saponins from soyabean. *Biosci. Biotechnol. Biochem.* **62**, 2291-2299.

Yoshiki Y, Okubo K. (1995) Active oxygen scavenging activity of DDMP (2,3-dihydro-2, 5-dihydroxy-6-methyl-4H-pyran-4-one) saponin in soybean seed. *Biosci. Biotechnol. Biochem.* **59**, 1556-1557.

Yuda Y, Tanaka J, Hirano F. (1999) Participation of Lipid peroxidation in rat pertussive vaccine pleurisy. III: Thiobarbituric acid (TBA) reactant and lysosomal enzyme. *Chem. Pharm. Bull.* **39**, 505-506.

Zentella PM. (1995) Blood glutathione in the alcoholic patient with hepatopathy. *Rev. Med. Hosp. Gen.* **58**, 52-58.