

Chronic ethanol consumption–induced hepatotoxicity and protective effect of *Boswellia serrata*

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Received December 24, 2015. Accepted January 5, 2016

ABSTRACT


Background: Chronic alcohol consumption is a known risk factor for liver disease, which represents a major cause of morbidity and mortality worldwide. The present experimental model was evaluated with medicinal plant *Boswellia serrata* and the results reflected potential beneficial and reducing risk factors for liver disease. **Aims and Objectives:** To determine the hepatoprotective activity of aqueous extract of *Boswellia serrata*. Chronic ethanol consumption is a major risk factor in determining liver disease and other metabolic syndrome via multiple mechanisms, including the regulation of the lipid metabolism. **Materials and Methods:** Male Wistar rats were divided into 6 groups ($n = 6/\text{group}$), and fed with a standard diet, ethanol, and ethanol supplemented with extracts for 6 weeks. The ethanol changes were determined via a serum enzyme profile of the liver. Liver toxicity was assessed in terms such as, serum indirect bilirubin, aspartate aminotransferase, alanine aminotransferase, total proteins, albumin, globulin, albumin: globulin ratio, and liver weight. **Results:** Oral administration of 250 and 500 mg/kg of Aqueous extract of *Boswellia serrata* offered a significant ($P < 0.01$) dose-dependent protection against ethanol-induced hepatotoxicity. **Conclusion:** This study revealed that aqueous extract of *Boswellia serrata* in phytochemical constituents plays an active role against enzymes' elevation and protection of liver.

KEY WORDS: *Boswellia serrata*; Ethanol; Serum Enzymes; Hepatotoxicity; Hepatoprotective

INTRODUCTION

Alcoholic liver disease (ALD) is a major health problem worldwide. ALD typically progresses through the stages of alcoholic steatosis, alcoholic hepatitis, and alcoholic cirrhosis.^[1,2] Chronic alcohol consumption has been reported to induce oxidative stress via multiple mechanisms.^[3,4] The earlier research report has linked chronic alcohol consumption and a variety of pathological conditions ranging from simple intoxication to severe life-threatening pathological states.^[5] The pathological condition of alcohol-induced liver disease is

characterized by morphological changes with minimal injury to more advanced liver damage.^[3,6] Herbal drugs play a role in the treatment and management of various liver disorders.^[7] *Boswellia serrata* (frankincense) is a moderate to large branching tree (growing to a height of 12 feet) found in India, Northern Africa, and the Middle East. *B. serrata* has been used for a variety of therapeutic purposes,^[8] such as inflammation,^[9] arthritis,^[10] asthma,^[11] psoriasis,^[12] colitis,^[13] Crohn's disease, and hyperlipidemia.^[14] Boswellic acids selectively inhibit leukotriene synthesis^[15,16] by inhibiting 5-LOX in an enzyme-directed, non-redox, noncompetitive mechanism via binding to pentacyclic triterpene-selective binding site.^[15,17] The *B. serrata* contains flavonoids and polyphenols as the major bioactive components.^[18,19] According to the WHO as many as 4 billion people or 80% of the earth's population are estimated to use some form of herbal medicine in their health care.^[20] *Boswellia serrata* extract has also been reported for anticarcinogenicity in mice with Ehrlich Ascites carcinoma and S-180 tumor by inhibiting the cell proliferation and growth inhibition due to the interference with biosynthesis of DNA,

Access this article online	
Website: http://www.njppp.com	Quick Response Code:
DOI: 10.5455/njppp.2016.6.24122015106	

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RNA, and proteins.^[21] In this study, we evaluated the influence of aqueous extract of *B. serrata* on liver-specific serum enzymatic activity associated with chronic ethanol consumption.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The leaves of *B. serrata* were collected from Paliyar tribes in Madurai district of Tamil Nadu. Botanical identification was carried out at the Department of Pharmacognosy, Ratnam Institute of Pharmacy, India. A voucher specimen No. RIP/2013/11(a) has been deposited in the museum of the Department of Pharmacy, Ratnam Institute of Pharmacy, India. The leaves were dried under shade, segregated, pulverized by a mechanical grinder, and passed through a 40-mesh sieve. The powdered plant materials were stored in an airtight amber-colored glass bottle.

Drugs and Chemicals

Enzyme kit was obtained from Span Diagnostics Ltd., Surat, India. Silymarin was purchased from Sigma-Aldrich, China. All other chemicals were of analytical grade procured from reputed Indian manufacturers.

Experimental animals

The experimental design was approved by the Institutional Animal Ethical Committee of Ratnam Institute of Pharmacy, India. Male Wistar rats 8–10 weeks old and weighing 180–200 g were kept under standard conditions of temperature ($25 \pm 5^\circ\text{C}$), relative humidity ($55 \pm 1\%$), and 12-h light and dark cycles; they were fed with standard pellet diet and water ad libitum.

Preparation of the Extracts

The dried powder was extracted sequentially by hot continuous percolation method using Soxhlet apparatus for 24 h. The solvent from the extracts was recovered under reduced pressure using a rotary evaporator and subjected to freeze drying in a lyophilizer until a dry powder was obtained.

Acute Toxicity Study

Acute oral toxicity study was performed as per Organization of Economic Cooperation and Development guideline 423. Each

group consists of three rats. Different doses are fed to the rats by oral intubation. The animals were observed individually every 30 min after dosing for the first 24 h, thereafter daily for 14 days. The time at which signs of toxicity appear/disappear was observed systematically and recorded for each animal.

Experimental Design

A total of 36 rats were equally divided into 6 groups of 6 each. Group I served as control without any treatment. Group II served as negative control. Animals of groups III, IV, and V were administered ethanol followed by Silymarin and different doses (250 and 500 mg/kg) of *B. serrata* for 6 weeks.

Group I: Control (10 mL/kg normal saline, bw p.o.)

Group II: Ethanol (15% v/v/kg bw p.o.)

Group III: Ethanol + Standard drug of Silymarin 100 mg/kg bw p.o.

Group IV: Ethanol + Aqueous extract of *B. serrata* 250 mg/kg bw p.o.

Group V: Ethanol + Aqueous extract of *B. serrata* 500 mg/kg bw p.o.

Histopathological Study

At the end of the study, all rats were euthanized by cervical dislocation after overnight fasting. Liver tissue was separated for further investigation. Liver slices were fixed in 10% formalin and embedded in paraffin wax. Sections of 5- μm thickness were made using a microtome and stained with hematoxylin-eosin. The sections were observed under a light microscope and photographs of each slide were taken at $40 \times$ magnification.

Statistical Analysis

Data are presented as mean \pm SEM ($n = 6$); statistical significance was calculated with one-way analysis of variance followed by Dunnett's t test. *P* value was found to be < 0.01 when the treated group was compared to the ethanol-treated group.

RESULTS

Acute Toxicity Study

After administration of 2,000 mg/kg dose of aqueous extract of *B. serrata*, the animals did not show a behavioral abnormality,

Table 1: Effect of AEBS on serum enzymes profile in control and experimental rats

Groups	IB	SGOT	SGPT	GLO	LW
I	0.60 \pm .10	256.66 \pm 2.45	89.16 \pm 1.77	2.83 \pm .07	5.23 \pm .17
II	1.60 \pm 0.00	472.16 \pm 2.38	167 \pm 2.40	5.22 \pm 0.14	7.94 \pm 0.01
III	0.67 \pm 0.01**	252.83 \pm 2.52**	93.5 \pm 2.56**	3.35 \pm 0.06**	4.87 \pm 0.02**
IV	0.87 \pm 0.00**	311 \pm 2.9**	100.66 \pm 2.60**	3.24 \pm 0.08**	6.34 \pm 0.02**
V	0.66 \pm 0.01**	258.83 \pm 2.25**	83.33 \pm 2.55**	2.72 \pm 0.08**	5.02 \pm 0.02**

Data are presented as mean \pm SEM ($n = 6$), statistical significance was calculated with one-way analysis of variance followed by Dunnett's t test.

***P* < 0.01 when treated group compared to ethanol-treated group.

Table 2: Effect of AEBS on serum enzymes profile in control and experimental rats.

Groups	TP	ALB	AGR
I	6.3 ± 0.11	3.75 ± 0.09	1.32 ± .00
II	4.44 ± 0.03	0.55 ± 0.08	0.10 ± 0.10
III	5.87 ± 0.02**	3.22 ± 0.09**	0.95 ± 0.01**
IV	5.16 ± 0.01**	2.52 ± 0.07**	0.77 ± 0.00**
V	6.38 ± 0.11**	3.25 ± 0.04**	1.19 ± 0.02**

Data are presented as mean ± SEM (n = 6), statistical significance was calculated with one-way analysis of variance followed by Dunnett's t test.

**P < 0.01 when treated group compared to ethanol-treated group.

dyslipidemia, sign of toxicity, or mortality in rats. Hence, *B. serrata* at doses of 250 and 500 mg/kg, p.o. was selected for further pharmacological investigations.

Pharmacological Interventions on Liver Enzymes Profile

The ethanol treatment for 6 weeks caused a significant increase in serum indirect bilirubin (IB), aspartate aminotransferase, alanine aminotransferase, globulin (GLO), and liver weight (LW). Chronic ethanol consumption rats showed decrease in total protein (TP), albumin (ALB), albumin: globulin ratio (A: G), and when, compared to rats fed with a standard diet. Treatment with AEBS 250, 500 mg/kg and, the Silymarin 100 mg/kg showed a significant (P<0.01) response against ethanol induced hepatic damage.

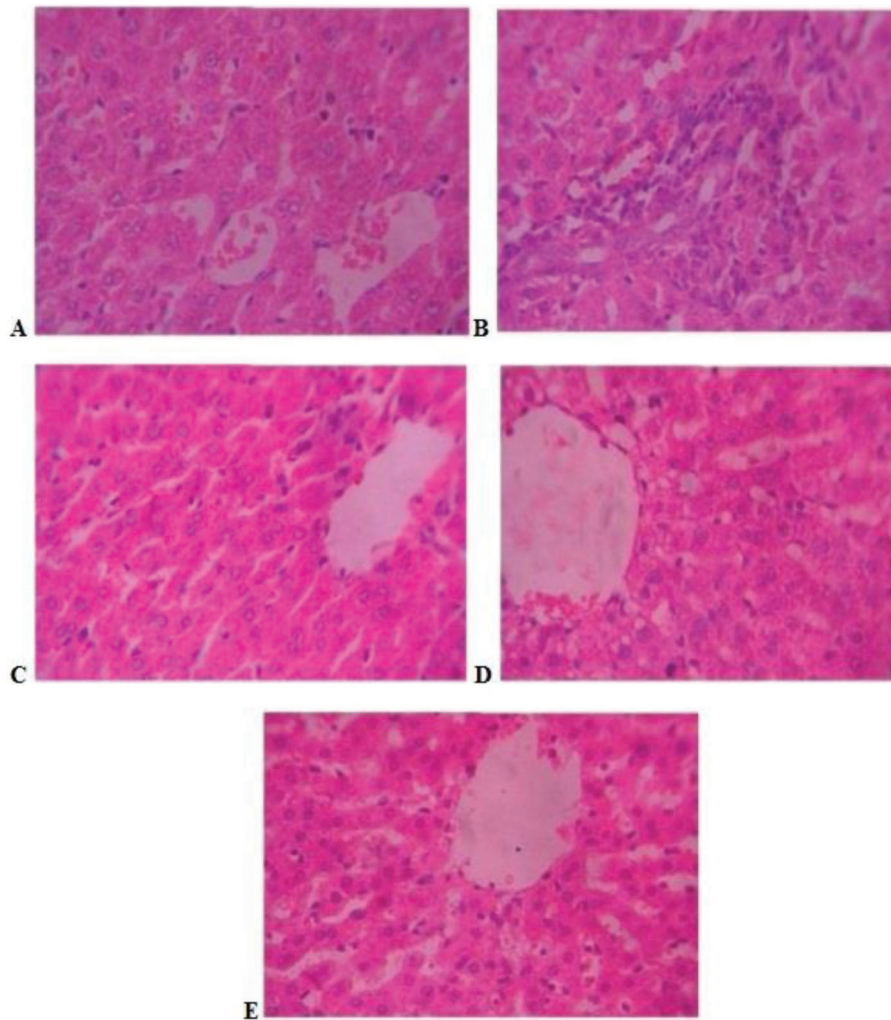


Figure 1: (A) Normal Control (the control animal shows normal architecture of liver cell), (B) negative control (histopathological studies revealed centrilobular and focal necrosis and ballooning in livers of mice challenged with ethanol), (C) Standard (standard drug-treated liver section revealing relatively normal), (D) AEBS 250 mg/kg (higher magnification of control rat liver section revealing swollen hepatocytes with decreased sinusoidal spaces), and (E) AEBS 500 mg/kg (high-dose rat liver section revealing comparatively normal hepatic parenchyma).

DISCUSSION

Previous report demonstrated that alkaloids,^[22] tannins,^[13] flavonoids,^[23] amino acids,^[24] and glycosides^[25] may protect hepatic damage.

Our results indicated that the phytochemical constituents of AEBS play an important role in hepatoprotective activity. The acute toxicity studies of *B. serrata* until 2,000 mg/kg were found to be nontoxic and did not cause any death of the test animals. Several epidemiological studies have shown flavonoid intake with a low risk of liver toxicity.^[26,27] Previous studies showed that flavonoids have antioxidative activity; free radical scavenging capacity; ability to prevent coronary heart disease; and hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities.^[27,28] In plant system, flavonoids help in combating oxidative stress and act as growth regulators.^[29,30]

As shown in Table 1 ethanol treated rats increased in serum activities compared to normal control rats. An increase in the liver enzymes may attribute to the damaged structural integrity of the liver, which results in the leakage of these enzymes from the cytosol into the bloodstream.^[31] In this study, proven different doses of AEBS markedly decreased IB level ($P < 0.01$) when compared to the ethanol-treated rats. Sindhu et al.^[32] reported that long-term alcohol consumption does not only activates free radical generation but also alters the levels of both enzymatic and nonenzymatic endogenous antioxidant systems. This results in oxidative stress with a cascade of effects affecting both functional and structural integrity of cell and organelle membranes.^[33]

Our investigation shows ethanol-treated rats had significant association with cellular necrosis and an increase in serum levels of many biochemical markers such as SGOT and SGPT.^[34] Studies by Tatiya et al. have found that the oxidative stress is highly correlated with a wide variety of inflammatory and metabolic diseases.^[35]

Consistently, we found that AEBS decreased serum globulin and LW. The previous study demonstrated that elevation of these biochemical enzymes caused damage in hepatocytes.^[36-38] We found in ethanol-treated rats a decrease in the level of serum TP, albumin, and A:G ratio (Table 2), and earlier studies revealed this effect.^[39-41] Kim et al. reported that hepatic steatosis is a common consequence of obesity, and its prevalence has been further characterized with hepatic fat accumulation.^[42] Ethanol can also sensitize cells, causing cell populations downstream of inflammatory cytokine signaling to respond more robustly.^[43] Present findings demonstrated that AEBS reversed the effect of ethanol-induced liver damage (Figure 1).

CONCLUSION

Our study showed that chronic intake of ethanol ameliorated hepatic damage in experimental rats. We found that among the dose group of AEBS, 250 and 500 mg/kg had a potential effect on inhibiting the progression of hepatotoxicity in ethanol-fed

rats. The results reflected may have beneficial and reducing risk factors for liver disease.

Acknowledgments

We thank the Department of Pharmaceutical Sciences, OPJS University, for providing the necessary facilities for carrying out the research work.

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How to cite this article: Subramaniyan V, Middha A. Chronic ethanol consumption-induced hepatotoxicity and protective effect of *Boswellia serrata*. *Natl J Physiol Pharm Pharmacol* 2016;6:170-174

Source of Support: Nil, **Conflict of Interest:** None declared.