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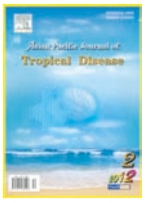

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Hepatoprotective activity of methanolic extract of *Barleria montana* leaves in ethanol treated rats

Shanaz Banu[1*], Arunachalam G[2], Jayaveera KN[3], Ashoka Babu VL[4], Vimal Kumar[5]

[1] *Dayananda Sagar College of Pharmacy, Kumaraswamy layout, Shavige Malleswara Hills, Bangalore -560 078*
[2] *PGP College of Pharmaceutical sciences and Research Institute, Namakkal-637 207, Tamil Nadu*
[3] *Jawaharlal Nehru Technological University, Anantapur, Andhra Pradesh*
[4] *M.S.Ramaiah College of Pharmacy, M.S.Ramaiah Nagar, M.S.I.T Post, Bangalore. Mob*
[5] *Dayananda Sagar College of Pharmacy, Rajiv Gandhi University of Health Sciences, Bangalore*

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ABSTRACT

Objective: The present study was undertaken to investigate the protective effect and possible mechanism of methanolic extract of *Barleria montana* (BM) on ethanol-induced rat hepatic injury. **Method:** This respective activity was assessed through monitoring liver function tests through the measurement of triglycerides, cholesterol, total protein, total bilirubin, serum enzymes like SGOT and SGPT and *in vivo* antioxidant parameters like lipid peroxidase, Superoxide dismutase(SOD) and catalase,. Further, hepatic tissues were also subjected to histopathological studies. **Result:** Pretreatment of BM methanolic extract (500mg/kg) reduced the fatty liver symptoms and significantly ($p<0.001$) inhibited the increase of respective serum enzyme levels. **Conclusions:** The results of the present study indicated that BM methanolic extract possess hepatoprotective effects which could act as an effective treatment for acute hepatic diseases.

1. Introduction

Liver is the largest glandular organ of the body. It plays an astonishing array of vital functions in the maintenance and performance of the body. Some of these major functions include carbohydrate, protein, and fat metabolism, detoxification and secretion of bile juice. Today, with the extensive use of hepatotoxicants in daily routine life, it has become imperative to safeguard human populations inhabiting poverty against liver diseases because mammalian liver is a highly toxicity sensitive organ and responsible for drug metabolism. Alcohol abuse is one of the major health problems worldwide.

The *Barleria montana*, family Acanthaceae, is an erect, unarmed undershrubs. Leaves are obovate, ovate-lanceolate, entire with purple coloured flowers. Traditionally the leaves of this plant is being used as hepatoprotective, antioxidant, antidiabetic, treatment of wounds and cuts etc.

The present study was undertaken to evaluate the hepatoprotective activity of this plant in experimental animal. The plant contains amongst many others alkaloids, flavonoids, phytosterols and phenolic compounds.

2. Materials and Method

2.1. Drugs and Chemicals

Silymarin was obtained from Micro labs, Bangalore. All the biochemical estimations were conducted at Dayananda Sagar College of Pharmacy using the Semi autoanalyser and all the solvents used were of analytical grade.

2.2. Plant material and Extracts

The leaves were collected from the Tirupati hills, Andhra Pradesh, during February-March of 2009, were

*Corresponding author: Dayananda Sagar College of Pharmacy, Rajiv Gandhi University of Health Sciences, Kumaraswamy layout, Shavige Malleswara Hills, Bangalore 560 078.
Tel: 9945640087,
E-mail: shanaz2906@gmail.com

authenticated by a botanist Dr. Madhavachetty, Professor of Botany, S.V.University, Tirupati.

2.3. Preparation of methanolic extract of leaves of Barleria montana

The leaves were air dried and coarsely powdered to 40 mesh and stored in air tight container till further use. Drug was defatted with petroleum ether and exhaustively extracted with methanol in soxhlet apparatus and the solvent was evaporated under reduced pressure and used for the activity.

2.4. Animals used

Wistar albino rats of either sex weighing between 150-200g were taken for the study. They were housed in polypropylene cages and maintained at (24±2) °C under 12 h light / dark cycle and they were fed *ad libitum* with standard pellet diet and had free access to water. They were initially acclimatized for the study and protocol was approved by the Institutional animal ethics committee as per the requirements of the committee for the purpose of control and supervision on animals (CPCSEA), New Delhi.

2.5. Experiment

2.5.1. LD_{50} Determination

Acute oral toxicity was estimated by using albino rats (150-200 g each) of both sex, were maintained in the animal house of the Department of Pharmacology, under standard conditions (temperature 25± 2℃, relative humidity 75 ± 5% and 12-h light and dark cycle). The animals had access to standard laboratory feed and water *ad libitum*. All procedures involving animals were performed in accordance with the OECD guideline 425[1]. The animals were fasted for 3 hours prior to the experiment and were administered with single dose of extract dissolved in 2% w/v Tween 80 and observed for mortality upto 48 h (short term toxicity). Based on short term toxicity, the dose of next animal was determined. All the animals were observed for long term toxicity (14 days) and LD_{50} was calculated. Experimental procedures were also examined and approved by internal ethical committee for animal welfare.

2.5.2. Hepatoprotective Activity

The hepatoprotective activity was carried out as described by Samuel Udem *et al.*[2] Albino rats of either sex were selected and divided into seven groups of six animals each. The animals were pretreated twice daily with vehicle (2% w/v Tween 80), BM leaf extract (250 and 500 mg/kg, silymarin (100mg/kg) orally, 1 h before ethanol administration. All the animals except normal control group received ethanol (3.76gm/kg *p.o.*) twice daily for a period of 25 days. On the 26th day, the animals were anaesthetised using anaesthetic ether and blood collected by retro orbital puncture. The levels of SGOT, SGPT, total bilirubin, cholesterol, triglycerides, total proteins and albumin[4] were estimated as per standard procedures. Immediately after collection of blood, the animals were euthanized with an overdosage of ether, livers were removed and kept in cold conditions. It was cross chopped with surgical scalpel into fine slices in chilled 0.25M sucrose, quickly blotted on a filter paper. The tissue was minced and homogenized in 10mM Tris-HCl buffer, pH 7.4(10% w/v) with 25strokes of tight Teflon pestle of glass homogenizer at a speed of 2500 rpm. The clear supernatant was used for oxidative stress markers assays like lipid peroxidation[3], reduced glutathione[6], superoxide dismutase and catalase[7]. Histopathology of liver was carried out by a modified method of Luna[9]. In brief, the autopsized livers were washed in normal saline and fixed in 10% formalin for 2 h followed by bovine solution for 6 h. The livers were then paraffin embedded and 5 μ thick microtome sections were made, processed with alcohol-xylene series and stained with haematoxylin. It was then studied under light microscope for any histological protection or damage.

2.5.3. Statistical analysis

The data obtained are expressed as mean ± SEM. The statistical differences between the means of various estimations were determined by One-way ANOVA. The values of $P < 0.05$ is been considered as significant.

3. Results

Preliminary phytochemical studies indicated the presence of alkaloids, carbohydrates, phytosterols, phenolic compounds and flavanoids. BM was found to be non toxic upto a dose of 5000 mg/kg.

Ethanol administration resulted in significant elevation of serum enzymes like SGPT and SGOT, triglycerides, cholesterol, total blilirubin while total protein was found to be decreased compared to normal control group (Table 1, Figure 1-6). *In vivo* antioxidant parameters like catalase, lipid peroxidation and SOD were studied and it was found to be decreased compared to normal control group (Table 2, Figure 7-9).

Pretreatment with silymarin and BM leaf extract significantly prevented the biochemical changes induced by ethanol (Fig No. 10).

Hepatocytes of normal control group showed a normal lobular architecture of liver. In the ethanol treated group the liver showed microvascular fatty changes, partially effaced

Table 1. Effects of extract on SGPT, SGOT, Triglycerides, cholesterol, Total protein, and Total bilirubin.

Group	SGPT	SGOT	Triglycerides	Cholesterol	Total blilirubin % mg	Total protein
Normal	30.19 ±0.5297	34.77±0.6114	85.31±2.292	142.87±2.876	0.212±0.01538	5.71±0.4010
Ethanol Treated	122.295±1.628	176.34±1.378	196.47±5.204	300.92±7.407	1.292±0.1754	3.25±0.2105
Silymarin + Ethanol	51.392±1.237 [a]	85.6317±0.9711 [a]	133.52±1.869[a]	180.13±4.009 [a]	0.378±0.03482 [a]	5.37±0.4374 [b]
Barleria montana leaf extract(250mg) + Ethanol	90.785±0.8743[a]	122.24±1.509 [a]	162.64±1.790 [a]	228.28±6.040 [a]	0.560±0.04513 [a]	3.63±0.3938 [ns]
Barleria montana leaf extract(500mg)	75.932±0.7407 [a]	110.7383±0.7939 [a]	144.98±2.104 [a]	194.12±3.672 [a]	0.408±0.02485 [a]	5.64±0.2560 [a]

Values are expressed as mean±S.E.M 9 (n = 6)
[a]p<0.001compared to ethanol intoxicated group, [b]p<0.01 compared to ethanol intoxicated group and [c]p<0.05 and ns>0.05 using 1 way ANOVA followed by Tukey Kramer Multiple comparison test.

Table 2. Effects of extract on liver catalase, SOD & Lipid peroxidation.

	Catalase	SOD	Lipid peroxidation
Normal	88.33±2.132	13.10±0.4342	5.83 ±0.3673
Ethanol Treated	31.18±1.231	4.17±0.1751	8.16± 0.3364
Silymarin	75.85±2.142 [a]	8.78±0.2611 [a]	6.11±0.2458 [a]
Methanolic extract of *Barleria montana* (250mg/kg)	41.095±2.411[c]	6.15±0.3342 [a]	7.44± 0.3179ns
Methanolic extract of *Barleria montana* (500mg/kg)	60.825±2.188 [a]	7.91±0.2423 [a]	5.06± 0.2051 [a]

Values are expressed as mean±S.E.M 9 (n = 6)
[a] P<0.001compared to ethanol intoxicated group, [b]P<0.01 compared to ethanol intoxicated group and [c]P<0.05 and ns>0.05 using 1 way ANOVA followed by Tukey Kramer Multiple comparison test.

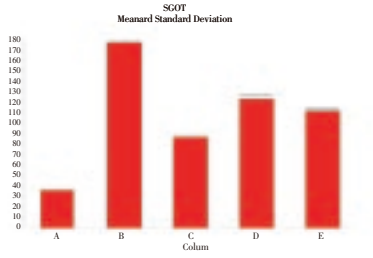


Figure 1: SGOT

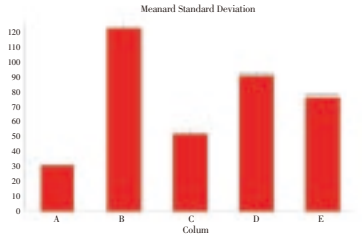


Figure 2: SGPT

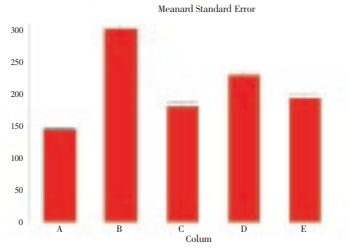


Figure 3: Cholesterol

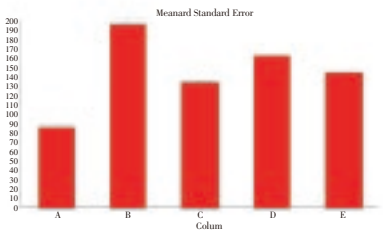


Figure 4: Triglycerides

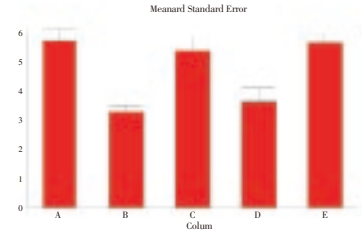


Figure 5: Total Protein

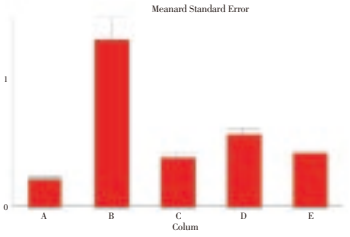
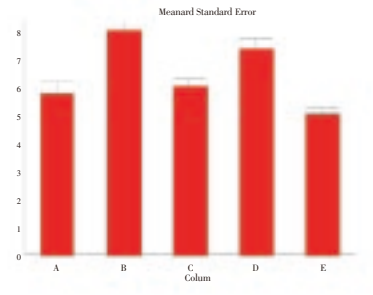


Figure 6: Total Bilirubin



FFigure 7: Lipid Peroxidase

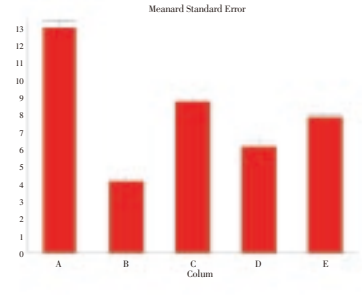


Figure 8: SOD

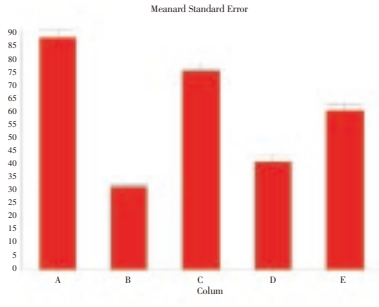


Figure 9: Catalase

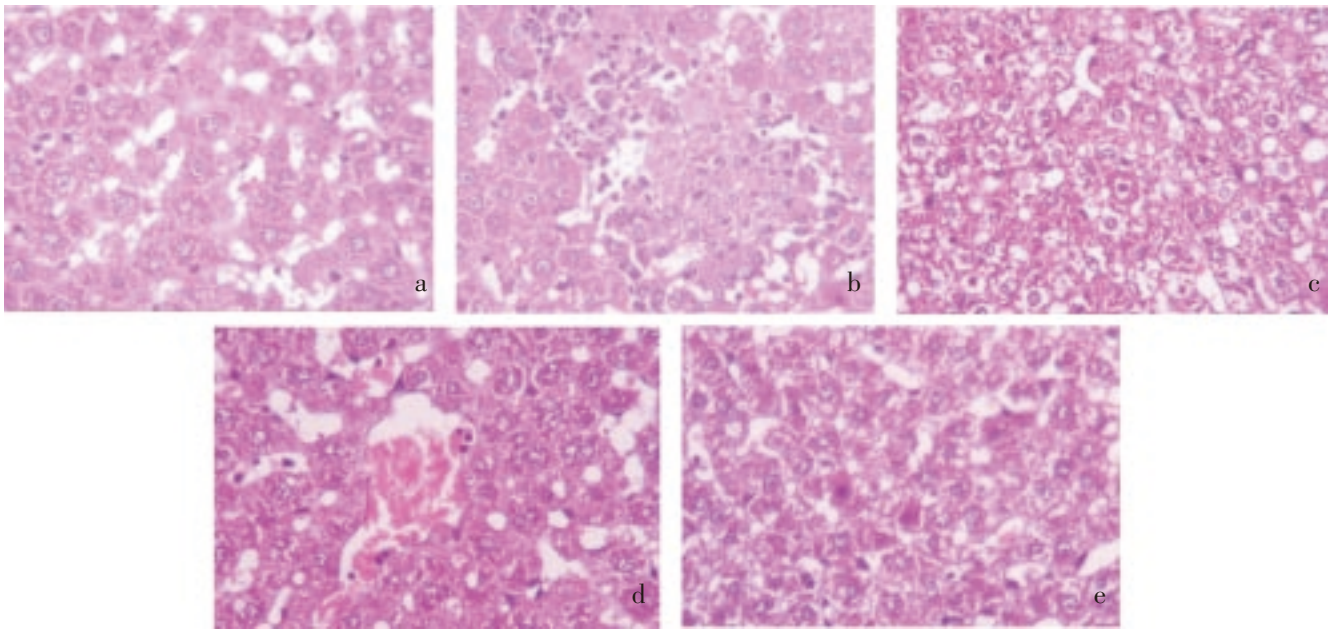


Figure 10.
a: Std Silymarin (100 mg/kg): Congestion of sinusoids, periportal mononuclear inflammatory infiltration; b: Alcohol toxicant. Partially effaced architecture, some of the hepatocytes show degenerative changes, epithelioid granulomas, aggregates of mononuclear inflammatory cells. Some of the sinusoids show congestion. Most of the hepatocytes show degenerative changes. There are seen epithelioid granulomas and aggregates of mononuclear inflammatory infiltration within the parenchyma; c: Normal saline:Section studied shows liver parenchyma with intact architecture. Most of the perivenular (zone-3) hepatocytes, periportal (zone-1) hepatocytes and midzonal (zone-2) hepatocytes appear normal. Within the hepatic parenchyma are seen few scattered mononuclear inflammatory cells; d: *Barleria montana* (250 mg/kg): Intact architecture, apoptotic and regenerative hepatocytes, sinusoidal congestion, aggregates of histiocytes are seen. Some of the sinusoids show congestion. Also seen are scattered apoptotic and regenerative hepatocytes. Intervening the hepatocytes are seen aggregates of histiocytes and mononuclear inflammatory cells; e: *Barleria montana* (500mg/kg): Intact architecture, few regenerative hepatocytes, sinusoidal congestion. Most of the sinusoids and central veins appear dilated and congestion. Also seen are scattered regenerative hepatocytes (Long arrow). Intervening the hepatocytes are seen scanty scattered mononuclear inflammatory infiltration.

architecture, some of the hepatocytes showed degenerative changes, epithelioid granulomas, aggregates of mononuclear inflammatory cells.

Silymarin pretreated groups and BM leaf extract treated groups showed minimal fatty changes and their lobular architecture was normal, showing that BM leaf extract have significant hepatoprotective activity.

4. Discussion

Liver is the major organ of our body. It can be injured by many chemicals and drugs. Here in the present study ethanol was used as a toxicant to induce liver damage, since it is clinically very relevant. Ethanol produces a constellation of dose related deleterious effects in liver. In chronic alcoholics, hepatomegaly occurs due to accumulation of lipids and proteins in hepatocytes with an impaired protein secretion by hepatocytes. During hepatic damage, cellular enzymes like Serum transaminases present in the liver cells leak into the serum resulting in increased concentrations. Ethanol administration for 25 days increased all these serum enzymes whereas administration of methanolic extract of *Barleria montana* showed significantly reduced Serum transaminase enzyme levels and increased total protein and albumin levels, indicating their hepatoprotective effect against alcohol-induced liver cell damage.

The benefits of BM methanolic extract has been further confirmed by histopathological observations. It was well-established that overdoses of ethanol lead to Partially effaced architecture, most of the hepatocytes showed degenerative changes, epithelioid granulomas, aggregates of mononuclear inflammatory cells. shrinkage of centrilobular reticular fibers, macrovesicular steatosis with ballooning of hepatic cells (fatty liver). Fatty change is characterized by the accumulation of triglyceride in hepatocytes. The three main mechanisms which may play a role in the development of alcoholic fatty liver are; increased substrate supply for fatty acid esterification, direct stimulation of the esterification pathway and decreased export from the liver of triglyceride as Very-Low-Density Lipoproteins (VLDL)[18]. These effects have been significantly reduced with the pretreatment of BM methanolic extract. The macrovesicular inflammation evoked by ethanol considerably decreased following extract pretreatment. Thus the accelerated recovery of hepatic cells by the BM methanolic extract was evidenced by histopathological observation, which suggests protection against membrane fragility, thus decreases the leakage of the marker enzymes into the circulation.

The results suggest that the flavanoid compounds in BM

methanolic extract play a pivotal role in the therapeutics of hepatotoxicity by increasing the body's natural antioxidant defenses with depletion in the ethanol-induced oxidative stress and reduction in the elevated levels of liver enzymes. The present investigation has opened avenues for further research in the development of potent phytomedicine for hepatoprotection from BM methanolic extract.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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References

[1] OECD 2001-guideline on acute oral toxicity (AOT), Environmental Health and Safety monograph series on testing and adjustment. No. 425.

[2] Samuel Udem, Innocent Nwaogu, Obinna Onyejekwe. Evaluation of Hepatoprotective activity of aqueous leaf extract of Swietenia Mahogani (Maliaceae) in Chronic Alcohol-Induced Liver Injury in Rats. *Macedonian Journal of Medical Sciences*, 2011; **4**(1):31-36.

[3] Anita Pal, Bhaskar Banerjee, Tanushree Banerjee, Manisha Masih, and Kailash Pal. Hepatoprotective activity of Chenopodium album Linn. plant against Paracetamol induced hepatic injury in rats, *Int J Pharm Sci* 2011;**3**(3): 55-57.

[4] Mihir Y Parmar, Purvi Shah, Vaishali Thakkar, Tejal R Gandhi. Hepatoprotective activity of Amomum subulatum Roxb against ethanol-induced liver damage. *Int. J. Gr. Pharm* 2009;**3**(3): 250-254

[5] Singha PK, Roy S, Dey S. Protective activity of andrographolide and arabinogalactan proteins from Andrographis paniculata Nees against ethanol-induced toxicity in mice. *J Ethnopharmacol* 2007;**111**:13-21.

[6] Arun.K, Balasubramanian.U, Comparative study on Hepatoprotective activity of Aegle marmelos and Eclipta alba against alcohol induced in albino rats. *Intl J. Env.Sci* 2011; **2**(2):389-402.

[7] Slater TF, Sawyer BC: The stimulatory effects of carbon tetrachloride and other halogenoalkanes or peroxidative reactions in liver fractions in vitro. *Biochem J* 1971; **123**: 805-814.

[8] Moran MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione Stransferase activities in rat lung and liver. Biochemica et Biophysica ACTA 1979; 582-67.

[9] Vipul Gujrati, Nilesh Patel, Venkat.N. Rao, K.Nandakumar, T.S.Gouda.Hepatoprotective activity of alcoholic and aqueous extract of leaves of Tylophora indica, *Indian Journal of Pharmacology* 2007;**39**(1): 43-47.

[10] Das S, Varadhen D, Mukherjee S, Vasudevan DM, Effects of chronic ethanol consumption in blood: a time dependant study on rat. *Indian J Clin Biochem* 2009; **24**(3): 301-6.

[11] Sutha Devaraj1, Sabariah Ismail1*, Surash Ramanathan1, Santhini Marimuthu2 and Yam Mun. Evaluation of the hepatoprotective activity of standardized ethanolic extract of Curcuma xanthorrhiza Roxb. *J. Med. Plant. Res* 2010; **4**(23): 2512-2517.

[12] Singha PK, Roy S, Dey S. Protective activity of andrographolide and arabinogalactan proteins from Andrographis paniculata Nees against ethanol- induced toxicity in mice. *J Ethnopharmacol* 2007,**111** : 13-21.

[13] Khandelwal KR. Practical Pharmacognosy Techniques and Experiments, 16th ed, Pune: Nirali Prakashan 2006.p. 149-56.

[14] Stickel F, Schuppan D. Herbal medicine in the treatment of liver diseases. *Digestive and Liver Disease* 2007; **39**: 293-304.

[15] Singanan V, Singanan M, Begum H. The Hepatoprotective Effect of Bael Leaves (Aegle marmalos) in alcohol induced liver injury in albino rats. *Int. J. Sci.Technol*. 2007; **2**(2): 83-92.

[16] Rosa MP, Gutiérrez, Rosario VS. Hepatoprotective and inhibition of oxidative stress of Prostechea michuacana. *Rec Nat Prod* 2009; **3**(1): 46-51.

[17] Murugaian P, Ramamurthy V, Karmegam N. Hepatoprotective activity of Wedelia calendulacea L. aainst acute hepatotoxicity in rats. *Res J Agri & Biol Sci* 2008; **4**(6): 685-687.

[18] Sutha Devaraj, Sabariah Ismail, Surash Ramanathan, Santhini Marimuthu and Yam Mun Fei. Evaluation of the hepatoprotective activity of standardized ethanolic extract of Curcuma xanthorrhizaRoxb.*J of Med Pl Res* 2010; **4**(23): 2512-2517.