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

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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 hepatotoxins 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
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 150–200 g 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°C, 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 up to 48 h (short term toxicity). Based on short term toxicity, the dose of next animal was 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 were determined 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 25 strokes 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.

2.5.2. 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 were determined 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 25 strokes 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 up to a dose of 5000 mg/kg.

Ethanol administration resulted in significant elevation of serum enzymes like SGPT and SGOT, triglycerides, cholesterol, total bilirubin 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.

<table>
<thead>
<tr>
<th>Group</th>
<th>SGPT</th>
<th>SGOT</th>
<th>Triglycerides</th>
<th>Cholesterol</th>
<th>Total bilirubin</th>
<th>Total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>30.19±0.5297</td>
<td>34.77±0.6114</td>
<td>85.31±2.292</td>
<td>142.87±2.876</td>
<td>0.212±0.01538</td>
<td>5.71±0.4010</td>
</tr>
<tr>
<td>Ethanol Treated</td>
<td>122.29±1.628</td>
<td>176.34±1.378</td>
<td>196.47±5.204</td>
<td>300.92±7.407</td>
<td>1.292±0.1754</td>
<td>3.25±0.2105</td>
</tr>
<tr>
<td>Silymarin + Ethanol</td>
<td>51.39±1.237</td>
<td>85.63±1.971</td>
<td>133.52±1.869</td>
<td>180.13±4.009</td>
<td>0.378±0.03482</td>
<td>5.37±0.4374</td>
</tr>
<tr>
<td>Barleria montana leaf extract(500mg)</td>
<td>75.93±0.7407</td>
<td>110.73±0.7939</td>
<td>144.98±2.104</td>
<td>194.12±3.672</td>
<td>0.408±0.02485</td>
<td>5.64±0.2560</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E.M (n = 6).

<0.001 compared to ethanol intoxicated group, *p<0.01 compared to ethanol intoxicated group and **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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Catalase</th>
<th>SOD</th>
<th>Lipid peroxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>88.35±2.132</td>
<td>13.10±0.4342</td>
<td>5.83±0.3673</td>
</tr>
<tr>
<td>Ethanol Treated</td>
<td>31.18±1.231</td>
<td>4.17±0.1751</td>
<td>8.16±0.3364</td>
</tr>
<tr>
<td>Silymarin</td>
<td>75.85±2.142</td>
<td>8.78±0.2611</td>
<td>6.11±0.2458</td>
</tr>
<tr>
<td>Methanolic extract of Barleria montana (250mg/kg)</td>
<td>41.09±2.411</td>
<td>6.15±0.3342</td>
<td>7.44±0.3179ns</td>
</tr>
<tr>
<td>Methanolic extract of Barleria montana (500mg/kg)</td>
<td>60.82±2.188</td>
<td>7.91±0.2423</td>
<td>5.06±0.2051</td>
</tr>
</tbody>
</table>

Values are expressed as mean±S.E.M (n = 6).

*P<0.001 compared to ethanol intoxicated group, **p<0.01 compared to ethanol intoxicated group and ***p<0.05 and ns>0.05 using 1 way ANOVA followed by Tukey Kramer Multiple comparison test.
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 infiltration within the parenchyma. 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.
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