Hepatoprotective effect of Barringtonia acutangula (L.) Gaertn leaf extracts against CCl₄ induced hepatic damage

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ABSTRACT

Oral pretreatment of five extracts (water, methanol, acetone, ethanol and petroleum ether) of the leaves of Barringtonia acutangula (LWBA, LMBA, LABA, LEBA and LPEBA; 200mg/kg and 400mg/ kg, po) showed significant hepatoprotective activity against CCl₄ induced hepatotoxicity by decreasing the activities of serum marker enzymes and bilirubin and increasing the protein content in a dose dependent manner, which was confirmed by the histopathological examination. The in vitro antioxidant activities of the extracts as determined by the total phenolics and FRAP methods were significantly higher. Among the five extracts, water extract (LWBA) showed maximum hepatoprotective and antioxidant activities followed by methanol (LMBA), acetonitrile (LEBA), ethanol (LEBA) and petroleum ether (LPEBA) extracts respectively.

Key words: Antioxidant activity, Barringtonia acutangula, CCl₄, Hepatoprotective property, Silymarin

INTRODUCTION

Exposure of various organic compounds and drugs can cause cellular damages through metabolic activation of those compounds to highly reactive substances. Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury to it or impairment to its functions may lead to many implications on one’s health [1-4]. Management of liver disease is still a challenge to the modern medicine. In absence of a reliable liver protective drug in the modern medicine, plants play a significant role as raw materials for some important drug formulations. These plants have been considered to increase health benefits, longevity, improve immune system and body resistance against different infections and diseases. They also possess hepatoprotective property against different toxins and drugs induced hepatic disorders [5-8].

Barringtonia acutangula (L.) Gaertn is an important medicinal plant widely used in the preparation of Ayurvedic formulations. B. acutangula belongs to the family Barringtoniaceae is a medium sized tree found in India, Sri Lanka, N. Australia etc. In Sanskrit, it is called Hizzala. In Ayurveda, its root, leaves and fruits are used in the treatment of jaundice, liver disorders, stomach disorders, leprosy and splenic diseases since many centuries [9]. Earlier studies have shown that the extract of the plant possesses antimicrobial and pesticidal activity. The crude extracts from the stem bark of B. acutangula has antimicrobial activity [10]. Petroleum ether extract of bark of B. acutangula found to be an effective pesticide [10]. No systematic study has been reported on protective activity of Barringtonia acutangula in experimental animal models or hepatic disorders in human beings. Therefore, the present study has been designed to evaluate the hepatoprotective property of the leaf extracts on CCl₄ induced hepatotoxicity in rats and in vitro antioxidant activity of the leaf extracts of B. acutangula.

MATERIALS AND METHODS

Collection of plant material and preparation of extracts: The leaves of B. acutangula were collected from Kannathirtha, Kerala, during 2008. It was authenticated by Dept. of Dravyaguna, Alva’s Ayurveda Medical College, Moodbidri. A voucher specimen no. D.G 90 is deposited in Dept. of Dravyaguna, Alva’s Ayurveda Medical College, Moodbidri, Karnataka, India. The shade dried leaves of B. acutangula were powdered mechanically and subjected to Soxhlet extraction using four solvents such as methanol, acetone, ethanol and petroleum ether for 24hrs. The extracts were concentrated under reduced pressure at 40°C to yield a semisolid mass and further evaporated at room temperature and stored in refrigerator till use. Water extract was prepared by boiling dried leaf powder in distilled water for 45 minutes. The extract was filtered and concentrated in water bath. The percentage yield of extracts was calculated. The five extracts were referred as LWBA (water extract, yield-20.68%), LMBA (methanol, yield-13.81%), LABA (acetone, yield-4.68%), LEBA (ethanol, yield-12.57%), and LPEBA (petroleum ether, yield-2.11%). Suspension of the extracts was prepared using gum acacia and distilled water.

CHEMICALS:

All the chemicals and solvents were of analytical grade and procured from E. Merck (India) Ltd., Mumbai. Standard kits for AST, ALT, ALP, protein and total bilirubin were obtained from Span Diagnostics Ltd, India.

Experimental animals:

Healthy male Wistar albino rats, 8 - 10 weeks old, weighing about 150 – 200 g were used for the study. Animals were maintained under standard conditions (12 hr light/dark cycle; 25±2°C, 45–60 % RH) and they were fed standard rat feed (Kamadenu Agencies, Bangalore, India) and tap water ad libitum. All the animals were acclimatized to laboratory conditions for a week before commencement of experiments. All experimental protocols were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) prior to the initiation of the experiments and the care of the animals was taken as per the CPCSEA regulations.

Acute toxicity study:

The acute toxicity study was carried out in male Wistar albino rats (200 g body weight) as per OECD guidelines No. 425 [11]. The animals were fasted overnight and next day the water extract of the leaves of B. acutangula suspended in gum acacia was administered orally at different dose levels (2000 mg/kg and 4000mg/kg body weight). The animals were observed continuously for 3 hrs for behavioural changes and then every 30mins for next 3hrs, and finally death after 24 hrs. No adverse effect or mortality was detected in albino rats during the 24hr observation period.

Phytochemical screening:

Freshly prepared extracts (LWBA, LMBA, LABA, LEBA and LPEBA) of the leaves of B. acutangula were subjected to preliminary phytochemical screening for detection of major chemical constituents such as steroids, triterpenoids, saponins, alkaloids, terpenoids, phytosterols, tannins, resins etc [12].

Quantitative determination of in vitro antioxidant properties of the leaf extracts of B. acutangula:

The total phenolic content in the leaf extracts (LWBA, LMBA, LABA, LEBA and LPEBA) was estimated by Folin-Ciocalteu method [13]. To 0.2 ml of the extract, 2.8 ml of water and 0.5 ml of Folin-Ciocalteu (1:2 diluted) were added. The tubes were incubated at room temperature for 3 minutes. 2 ml of 20% Na₂CO₃ was added to the tubes and kept in boiling water bath for 1 minute and cooled. Their absorbance at 765 nm was measured using UV-Visible spectrophotometer. A standard curve was prepared using catechol. The values were expressed in mg/g dry sample. Total antioxidant activity (FRAP assay) was analyzed using a modified method of Benzie and Strain [14]. The stock solutions included 300 mM acetate buffer (46.3ml 0.2M acetic acid and 0.2M sodium acetate, pH 3.6, make
up to 100 ml, 10mM TPTZ (2,4,6-tripyridyl-5-triazine) solution in 40mM HCl and 20mM FeCl$_3$·6H$_2$O solution. The fresh working solution was prepared by mixing 25ml acetate buffer, 2.5mL TPTZ, 2.5 ml FeCl$_3$·6H$_2$O. Plant extracts (20µl) were added to react with 900µl of the FRAP solution. Readings of the coloured product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was prepared using FeSO$_4$. Results were expressed in mMols /g sample and compared with that of ascorbic acid (Table 2).

**Table 1: Phytochemical screening of various extracts of the leaves of B. acutangula (viz, LWBA, LABA, LMA, LABA and LPEBA)**

<table>
<thead>
<tr>
<th>Phytochemical constituents</th>
<th>Leaf Extracts</th>
<th>LWBA</th>
<th>LABA</th>
<th>LMA</th>
<th>LPEBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Resins</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ = Present, - = Absent

**Quantitative determination of in vitro antioxidant properties of the leaf extracts of B. acutangula**

Determination of total phenolic content in the five leaf extracts revealed that LWBA extract showed maximum total phenolics, followed by LABA, LMA, LWBA and LPEBA extracts. Among the five extracts LWBA showed maximum total antioxidant power, followed by LABA, LMA, LWBA and LPEBA extracts. The FRAP values of LWBA, LMBA, LABA and LPEBA were higher than that of ascorbic acid (Table 2).

**Table 2: Quantitative determination of total phenolics and total antioxidants in the leaf extracts of B. acutangula**

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Total phenolics (mg/g)</th>
<th>Total antioxidants (mMols/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LWBA</td>
<td>142.4</td>
<td>378.33</td>
</tr>
<tr>
<td>LABA</td>
<td>126.8</td>
<td>365.00</td>
</tr>
<tr>
<td>LMA</td>
<td>112.06</td>
<td>345.00</td>
</tr>
<tr>
<td>LPEBA</td>
<td>65.73</td>
<td>138.33</td>
</tr>
<tr>
<td>LPEBA</td>
<td>4.89</td>
<td>30.00</td>
</tr>
</tbody>
</table>

**Effect of leaf extracts on serum marker enzyme levels:**

Table 3 shows a significant increase in the levels of serum marker enzymes such as AST, ALT and ALP in CCl$_4$ treated animals. In contrast pretreatment with LWBA, LABA, LMA, LPEBA and silymarin (200 mg/kg, po) and silymarin (25 mg/kg, po) exhibited reduced hepatotoxicity by decreasing the levels of serum marker enzymes in a dose dependent manner (P < 0.05).

**Table 3: Effect of various extracts of the leaves of B. acutangula (viz, LWBA, LABA, LMA, LABA and LPEBA) on serum enzyme and biochemical parameters in CCl$_4$ induced hepatic damage in rats [Values are mean ± SE from 6 animals in each group]**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg po)</th>
<th>SAST (U/L)</th>
<th>SALT (U/L)</th>
<th>SALP (U/L)</th>
<th>Total protein (mg/dl)</th>
<th>Total bilirubin (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Vehicle control</td>
<td>5 ml</td>
<td>63.17 ± 1.14</td>
<td>57 ± 0.73</td>
<td>70 ± 0.58</td>
<td>7.70 ± 0.13</td>
<td>0.26 ± 0.02</td>
</tr>
<tr>
<td>II CCl$_4$ control</td>
<td>0.7 ml</td>
<td>350 ± 4.73</td>
<td>301 ± 5.04</td>
<td>317 ± 3.34</td>
<td>5.66 ± 0.10</td>
<td>3.04 ± 0.15</td>
</tr>
<tr>
<td>III CCl$_4$ + silymarin 25</td>
<td>500 mg/kg</td>
<td>105 ± 0.90</td>
<td>135 ± 0.90</td>
<td>154 ± 0.90</td>
<td>5.22 ± 0.07</td>
<td>0.97 ± 0.02</td>
</tr>
<tr>
<td>IV CCl$_4$ + LWBA 200</td>
<td>500 mg/kg</td>
<td>82.16 ± 0.95</td>
<td>150 ± 0.90</td>
<td>175 ± 0.90</td>
<td>5.10 ± 0.07</td>
<td>0.43 ± 0.01</td>
</tr>
<tr>
<td>V CCl$_4$ + LMBA 200</td>
<td>500 mg/kg</td>
<td>125 ± 0.91</td>
<td>150 ± 0.90</td>
<td>175 ± 0.90</td>
<td>6.14 ± 0.01</td>
<td>1.09 ± 0.01</td>
</tr>
<tr>
<td>VI CCl$_4$ + LABA 200</td>
<td>500 mg/kg</td>
<td>97.17 ± 0.91</td>
<td>122 ± 0.91</td>
<td>178 ± 0.91</td>
<td>6.38 ± 0.01</td>
<td>0.53 ± 0.01</td>
</tr>
<tr>
<td>VII CCl$_4$ + LEBA 200</td>
<td>500 mg/kg</td>
<td>126 ± 0.91</td>
<td>150 ± 0.91</td>
<td>178 ± 0.91</td>
<td>6.24 ± 0.01</td>
<td>0.55 ± 0.01</td>
</tr>
<tr>
<td>VIII CCl$_4$ + LPEBA 200</td>
<td>500 mg/kg</td>
<td>99.33 ± 0.91</td>
<td>125 ± 0.91</td>
<td>178 ± 0.91</td>
<td>6.10 ± 0.01</td>
<td>1.38 ± 0.01</td>
</tr>
<tr>
<td>IX CCl$_4$ + LPEBA 200</td>
<td>500 mg/kg</td>
<td>301.6 ± 0.91</td>
<td>317 ± 0.91</td>
<td>333 ± 0.91</td>
<td>5.75 ± 0.01</td>
<td>2.95 ± 0.01</td>
</tr>
</tbody>
</table>

One way ANOVA followed by Tukey’s multiple comparison post hoc test. *P<0.01 when compared with vehicle treated control group. *P<0.05, *P<0.01 when compared with CCl$_4$ treated control group.

**Effect of leaf extracts on biochemical parameters:**

In the CCl$_4$ group, there was a significant increase in total bilirubin and decrease in total protein content whereas pretreatment with LWBA, LABA, LMA, LPEBA (200 mg/kg and 400mg/kg, po) caused significant reduction in total bilirubin and increase in total protein content in a dose dependent manner (Table 3).
Histopathological study:
Histopathological observations basically supported the results obtained from serum enzyme assays. The liver sections of the CCl4 treated groups showed hepatic cells with severe fatty change. Pretreatment with LWBA, LMBA, LABA, LEBA and LPEBA (200 mg/kg and 400 mg/kg, po) and silymarin (25 mg/kg, po) exhibited significant liver protection against CCl4 induced liver damage, which is evident by the presence of more normal hepatocytes and reduced fatty change (Fig. 1a-e).

DISCUSSION
CCl4 induced hepatic injury is commonly used model for the screening of hepatoprotective drugs and extent of hepatic damage is assessed by the level of released cytoplasmic alkaline phosphatase and transaminase in circulation. Hepatocellular necrosis or membrane damage leads to very high levels of serum AST and ALT released from liver to circulation. Among the two, ALT is a better index of liver injury and it represents the 90% of total enzyme present in the body. The elevated levels of serum marker enzymes are indicative of cellular leakage and loss of functional integrity of cellular membrane in liver [21]. Studies have shown that various herbal extracts could protect organs against CCl4 induced oxidative stress by altering the levels of different serum marker enzymes.

In the present study, five extracts of leaves of B. acutangula (LWBA, LMBA, LABA, LEBA and LPEBA) at a dose of 400 mg/kg, po caused a significant inhibition in the levels of AST and ALT with respect to the normal range and this is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl4. On the other hand suppression of elevated ALP with concurrent depletion of raised bilirubin level and an increase in the total plasma protein content suggests the stability of biliary dysfunction in rat liver during hepatic injuries with toxicants [22]. These results indicate that LWBA, LMBA, LABA, LEBA and LPEBA extracts preserved the structural integrity of the hepatocellular membrane and liver cell architecture damaged by CCl4 which was confirmed by histopathological examination.

Preliminary phytochemical screening of the extracts (LWBA, LMBA, LABA, LEBA and LPEBA) of the leaves of B. acutangula showed the presence of alkaloids, glycosides, phytosterols, triterpenoids, tannins, phenolic compounds and flavonoids. Steroids and resins were found in LMBA, LABA and LPEBA extracts. Saponins were present only in LWBA extract. In the present state of knowledge of the chemical constituents of this plant, it is not possible to attribute the hepatoprotective effect to one or more these active principles detected in the leaf extracts of B. acutangula. However, flavonoid [23] triterpenoids [24], saponins [25] and alkaloids [26] are known to possess hepatoprotective activity in animals.

Total phenolics are the major plant compounds with antioxidant activity. This activity is believed to be mainly due to their redox properties [27], which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Results obtained in the present study revealed that the level of phenolic content in the leaf extracts of B. acutangula was considerable. The results strongly suggest that phenolics are important components of this plant. Some of its pharmacological properties could be attributed to the presence of these variable components. The antioxidant potentials of the leaf extracts of B. acutangula were estimated from their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) [24]. Antioxidant activity increased proportionally with the phenolic content. Studies have shown a highly positive relationship between total phenolics and antioxidant activity in many plant species [28].

It can be concluded that the five extracts of leaves of B. acutangula protects liver against oxidative damages and could be used as an effective protector against CCl4 induced hepatic damages. Further work is needed to fully characterize the responsible active principle(s) present in the plant and to elucidate its possible mode of action, which is in progress in our laboratory.

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