Hepatoprotective effect of *Barringtonia acutangula* Linn. leaves on carbon tetrachloride-induced acute liver damage in rats

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*Barringtonia acutangula* Linn. (Barringtoniaceae) is widely distributed throughout India, Southeast Asia, Australia, and Africa. It is used in various indigenous systems of medicine against several diseases and almost every part of the plant has diverse pharmacological properties. The present investigation includes a detailed *in vitro* and *in vivo* hepatoprotective activity study of the methanol extract of *B. acutangula* (BA) leaves on carbon tetrachloride (CCL4) with liquid paraffin (1:1) induced hepatic injury in rats. The methanol extract of BA showed significant (*P*<0.001) hepatoprotective activity at a dose of 3.3 mg/mL and 250 mg/kg when screened *in vitro* and *in vivo*, respectively. An optimized High Performance Thin Layer Chromatographic (HPTLC) fingerprint pattern has been also developed at 254 nm for the identification and quality assessment of raw leaves of (BA).

**Keywords:** *Barringtonia acutangula*, Carbon tetrachloride, Explant culture, Hepatoprotective activity, Liver Damage, Silymarin

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**Introduction**

In recent years, in spite of tremendous scientific advancement in the field of hepatology still liver problems are on rise. Jaundice and hepatitis are the most widespread and major hepatic disorders that account for high death rate1. In India about 20,000 deaths are found every year due to liver disorder. Hepatocellular carcinoma is one of the ten most common tumours in the world with over 2, 50, 000 new cases in each year2. In India, about 40 polyherbal commercial formulations are being used and reported to have hepatoprotective action. Extracts of different plants have also been reported to cure liver disorders3,4.

The medicinal plant, *Barringtonia acutangula* Linn. (Family-Barringtoniaceae) commonly known as *Hinjal* (Plate 1) is widely cultivated throughout India, Southeast Asia, Australia, and Africa5. Traditionally, its various parts of this plant (root, seed, bark and leaf) are used as carminative, expectorant, bitter tonic, emetic, fish poison and also in the treatment of seminal weakness, diarrhoea and gonorrhoea6. The plant is reported to have anti-implantation activity in female albino rats7. Seeds and leaves are useful in vitiated conditions of *pitta* and *kapha*, colic, intestinal worms, wounds, ulcers, skin diseases8, diarrhoea9, hallucinations9, as an anthelmintic, leprosy, splenomegaly, cough, bronchitis, dysmenorrhoea, intermittent fevers, opthalmitis, syphilis, cephalalgia and lumbago5. In view of excellent pharmacological activities and ethnomedicinal claim, it was decided to evaluate the medicinal properties of methnolic extract of leaves of the plant on carbon tetrachloride-induced acute liver damage in rats and the obtained results are presented herein.

**Materials and Methods**

**Plant material**

*B. acutangula* (BA) was collected from the coastal area of Jajpur district, Orissa, India in the month of September, 2007 and authenticated at Natural Product Department, IMMT, Bhubaneswar, Orissa and a voucher specimen (10001/RRL/B) was deposited in the herbarium. The leaves were shade dried and powdered with a mechanical grinder. The powder was passed through sieve number 40 and stored in an airtight container under laboratory condition (at 23 ± 4°C with 36 ± 3% of relative humidity) for further use.

**Extraction**

The air dried powdered material of leaves was extracted with methanol in Soxhlet apparatus10. The solvent was removed under reduced pressure and the dried extract was stored in vacuum dessicator (yield 12.6% w/w). The generated extract was subjected to evaluation of hepatoprotective activity and HPTLC study.

**Animals**

White albino rats (Wistar strain) weighing between 200–250 g of either sex were maintained under...
uniform laboratory conditions and provided with food and water *ad libitum*. The Institutional Animal Ethics Committee approved the experimental protocol and animals were maintained under standard conditions for an acclimatization period of 15 days before performing the experiment. The conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) vide registration no. 990/c/06/CPCSEA.

The acute oral toxicity study was done by the administration of BA extracts according to OECD guideline at dose range 100 to 5000 mg/kg, bw. BA extracts did not produce any mortality up to a dose of 5000 mg/kg.

**In vitro hepatoprotective activity**

Explant cultures of rat liver was done to evaluate the activity by growing cells in Dulbecco’s Modified Eagle’s Medium (DMEM) (Sigma, USA) with new born calf serum (NBCS) at 5% as per standard protocol. After 48 h of explant culture, CCl₄ (83.3 µL/mL) was added to the culture in duplicate. Similarly, CCl₄ and methanolic extracts (3.3, 1.65 and 0.825 mg/mL) of BA were added in duplicate wells and CCl₄ along with Silymarin (0.5 mL) (Silybon suspension, Micro Labs Ltd., Bangalore) was taken as standard. The plates were incubated for 2 h. After 2 h of incubation, the explant supernatant was collected for evaluating various enzymes like glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP). GOT and GPT were assayed by kits from Crest Biosoftware, India. Transaminases activities were measured as UV kinetic reaction using IFCC method. The absorbance of reaction was determined at 340 nm by spectrophotometer. The ALP activity was determined as visible kinetic reaction method at 405 nm.

**In vivo hepatoprotective activity**

Healthy albino rats were divided into 4 groups each containing 6 animals. Group I (Control) received normal saline (5 mL/kg). Group II (Toxic Control) received CCl₄ in liquid paraffin (1:1, 1 mL/kg body weight, i.p.). Groups III and IV received standard drug Silymarin (Silybon suspension, Micro Labs Ltd., Bangalore) (5 mL/kg, p.o) and methanol extract (250 mg/kg) once in a day and CCl₄ as mentioned above. Treatment duration was 8 weeks and the dose of CCl₄ was administered twice in a week. Animals were anaesthetized with ether and sacrificed 24 h after the last injection. Blood was collected by cardiac puncture, allowed to clot and serum separated and used for biochemical studies. The biochemical studies include serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, alkaline phosphatase, bilirubin (total and direct), serum triglycerides and total protein. The SGOT, SGPT and ALP was measured as mentioned above. The serum triglycerides levels were determined by the method of
The serum bilirubin was determined by the modified Jendrassik & Grof’s method. Total protein content was measured by Biuret method.

Statistical analysis

The values of the biochemical parameters are given in terms of mean ± SEM. The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Tukey’s test and P<0.05 was considered as statistically significant.

Development of HPTLC fingerprint

The HPTLC analysis of the extract was carried out with an aim to optimize the solvent system for chromatographic separation and to observe the number of components present in the said extract with optimized chromatographic condition. The sample solution for chromatography was prepared by dissolving 50 mg of crude extract in 25 mL of methanol. A 60 µL of sample solution was applied on Silica gel pre-coated aluminum sheet by using Linomat 5 applicator as 6 mm band. The plate was developed with acetone-hexane (4:6, v/v) as mobile phase and allowed to run up to 80 mm. The chromatograms were scanned densitometrically using a Camag TLC scanner 3 with winCATS software (version 1.3.4) in absorbance-reflectance scan mode at different wavelength and fingerprinting patterns were developed. The scanner slit was set at 5.0 × 0.45 mm (micro) with 20 mm/s scanning speed and 100 µm per step data resolution were used.

Results and Discussion

The results of in vitro hepatoprotective study exhibited from attachment and proliferation of explant cultures within 48 h and the supernatant subjected to GOT, GPT and the ALP estimation exhibited significant variations in all experimental sets (Table 1). In CCl₄ treated explants culture, GOT (35.61 ± 3.71 U/L) and GPT (21.94 ± 1.61 U/L) level increased significantly (P<0.001) when compared to control (12.55 ± 2.37 U/L). Methanol extract of BA in higher dose (3.3 mg/mL) significantly lower the GOT (20.76 ± 2.33, U/L) (P<0.01) level where as no significant changes were observed in GPT level (16.64 ± 1.73 U/L) when compared to CCl₄ treated explant culture sets. Methanol extract at the dose mentioned above significantly (P<0.001) decrease the ALP level (22.59 ± 2.28 U/L) when compared to CCl₄ treated toxic sets (38.3 ± 3.35 U/L). The SGPT level was decreased when the explant culture was treated with both the standard drug and extract however, the decrease in SGPT level was not significantly observed in the plant extract treated groups even at the highest dose (3.3 mg/mL). The result showed that with an increase in extract dose (0.825-3.3 mg/mL), resulted in lower SGPT values indicating that the pattern of lowering of SGPT is dose dependent.

The results of in vivo hepatoprotection exhibited by methanol extract of BA are tabulated in Table 2. As the in vitro hepatoprotective study showed the protective effect of the extract in dose dependent manner, the protective effect was repeated in vivo with a single dose to screen the efficacy of the extract over a specified duration of treatment (i.e, 8 weeks) only. Hence, 250 mg/kg bw dose was used for the study. Rats administered with CCl₄; liquid paraffin (1:1) dosage regimen developed significant hepatocellular damage as evident from significant elevation in serum activities of GOT, GPT, ALP, bilirubin concentration (total and direct), triglycerides and decrease in total protein compared to normal values. The methanol extract of BA leaves (250 mg/kg/day, P.O.) exhibited a significant

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Table 1—Effect of methanol extract of Barringtonia acutangula and Silymarin on explant cultures of rat liver (n=6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>GOT</th>
<th>GPT</th>
<th>ALP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12.55±2.37</td>
<td>2.72±0.47</td>
<td>14.19±1.4</td>
</tr>
<tr>
<td>CCl₄ (83.3 µL/mL)</td>
<td>35.61±3.71</td>
<td>21.94±1.61</td>
<td>38.3±3.35</td>
</tr>
<tr>
<td>CCl₄+Silymarin (1.9 mg/mL)</td>
<td>14.18±2.23**</td>
<td>9.02±1.86***</td>
<td>16.55±2.98***</td>
</tr>
<tr>
<td>CCl₄+ Extract (3.3 mg/mL)</td>
<td>20.76±2.33***</td>
<td>16.64±1.73**</td>
<td>22.59±2.28***</td>
</tr>
<tr>
<td>CCl₄+ Extract (1.65 mg/mL)</td>
<td>24.09±2.7***</td>
<td>16.92±2.23***</td>
<td>27.21±2.98 a, b***</td>
</tr>
<tr>
<td>CCl₄+ Extract (0.825 mg/mL)</td>
<td>26.94±2.17***</td>
<td>19.29±1.91***</td>
<td>29.52±1.62 c, ns</td>
</tr>
</tbody>
</table>

Values are given in mean ± SEM.

a - P<0.05, b - P<0.01, c - P<0.001 significantly different when compared to control;

*P<0.05, **P<0.01, ***P<0.001 significantly different when compared to CCl₄.

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SGOT- Serum glutamate oxaloacetate transaminase, SGPT- Serum glutamate pyruvate transaminase, ALP- Alkaline phosphatase
reduction ($P<0.001$) in CCl$_4$: liquid paraffin (1:1) induced increased serum levels of GOT, GPT, ALP, bilirubin concentration, and triglycerides where as the protein content increased significantly ($P<0.001$) thus reversing hepatotoxicity causing significant liver recovery.

HPTLC analysis was carried out only to resolve the number of components and to indicate the component present in significant level (RF 0.17, peak 3). The developed chromatogram represents HPTLC fingerprint of extract of BA leaves at optimized condition at 254 nm and consisting of eight well resolved peaks (Figure 1) which were taken for hepatoprotective study. It indicates, that a component at RF 0.17 (peak 3) in the chromatogram is present in significant level. The results revealed that the chromatographic fingerprint evaluation could be used efficiently for the identification, authentication and quality assessment of raw leaves of BA.

**Conclusion**

The results of our study suggest that the methanol extract of BA leaves exhibited significant ($P<0.001$) hepatoprotective activity at a dose of 3.3 mg/mL and 250 mg/kg when screened in vitro and in vivo, respectively. Further studies aimed at elucidation of exact mechanism, isolation and purification of active phytoconstituents with potent hepatoprotective activity.

**Acknowledgment**

The authors wish to thank the Head of the Department, University Department of Pharmaceutical Sciences, Utkal University for providing the necessary

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGOT (U/L)</th>
<th>SGPT (U/L)</th>
<th>ALP (U/L)</th>
<th>T. Bil (mg/dL)</th>
<th>D. Bil (mg/dL)</th>
<th>STG (mg/dL)</th>
<th>TP (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>111.17±4.75</td>
<td>73.05±15.58</td>
<td>120.67±7.65</td>
<td>0.80±0.09</td>
<td>0.12±0.02</td>
<td>93.26±5.45</td>
<td>7.78±0.83</td>
</tr>
<tr>
<td>II</td>
<td>314.14±10.95</td>
<td>268.98±20.65</td>
<td>320.95±17.54</td>
<td>2.77±0.3</td>
<td>0.98±0.03</td>
<td>234.32±19.95</td>
<td>2.55±0.37</td>
</tr>
<tr>
<td>III</td>
<td>136.15±17.8***</td>
<td>122.14±19.7***</td>
<td>143.04±20.7***</td>
<td>1.38±0.19***</td>
<td>0.15±0.058***</td>
<td>108.86±23.18***</td>
<td>6.72±0.66***</td>
</tr>
<tr>
<td>IV</td>
<td>205.13±27.23 a***</td>
<td>160.28±25.5***</td>
<td>192.33±22.11***</td>
<td>1.74±0.21***</td>
<td>0.28±0.048***</td>
<td>137.39±24.15***</td>
<td>6.19±0.91***</td>
</tr>
</tbody>
</table>

Values are given in mean ± SEM.
a - $P<0.05$, b - $P<0.01$, c - $P<0.001$ significantly different when compared to control;
*P<0.05, **P<0.01, ***P<0.001 significantly different when compared to CCl$_4$.
ns statistically not significant ($P>0.05$).

Group I (Control) received normal saline (5 ml/kg), Group II (Toxic Control) received CCl$_4$ in liquid paraffin (1:1, 1 ml/kg body weight, i.p.), Group III and IV received standard drug Silymarin (5 ml/kg, p.o.) and methanol extract BA (250 mg/kg), respectively once daily followed by CCl$_4$ administration.

SGOT- Serum glutamate oxaloacetate transaminase, SGPT- Serum glutamate pyruvate transaminase, ALP- alkaline phosphatase, T. Bil.- Total bilirubin, D. Bil- Direct bilirubin, STG- Serum triglycerides, TP- Total protein.

![Fig. 1—HPTLC chromatogram of Barringtonia acutangula at 254 nm](attachment:image.png)
facilities for carrying out this research work and to Dr. N. K Dhal, Natural Product Department, Institute of Minerals & Materials Technology for identification of plant species.

References