Protective effects of Flacourtia indica aerial parts extracts against paracetamol-induced hepatotoxicity in rats


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Abstract

The petroleum ether, ethyl acetate and methanol extracts of the aerial parts of Flacourtia indica (Burm. f.) Merr., were evaluated for hepatoprotective properties. In paracetamol-induced hepatic necrosis in rat models, all extracts were found to reduce serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and serum alkaline phosphatase (SAP). The most significant reduction of the serum level of SGOT and SGPT were exhibited by petroleum ether and ethyl acetate extracts at a single oral dose of 1.5 g/kg of body weight with a reduction of 29.0% SGOT & 24.0% SGPT level by petroleum ether extract, and 10.57% SGOT & 6.7% SGPT level by ethyl acetate extract compared to paracetamol (3 g/kg of body weight) treated animals. Histopathological examination also showed good recovery of paracetamol-induced necrosis by petroleum ether and ethyl acetate extracts. On the other hand, the methanol extract obtained by successive cold extraction did not show any remarkable effect on paracetamol-induced hepatic necrosis. The hepatoprotective effects exhibited by petroleum ether and ethyl acetate extract might be mediated through the inhibition of microsomal drug metabolizing enzymes.

Keywords: Flacourtia indica; Flacouriaceae; paracetamol; hepatoprotective; serum glutamic oxaloacetic transaminase (SGOT); serum glutamic pyruvic transaminase (SGPT).
1. Introduction

*Flacourtia indica* (Burm. f.) Merr. synonymous to *Flacourtia ramontchi* L’Herit. (Family-Flacouriaceae), commonly known as ‘Baichi’ or ‘Katai’, is an indigenous medicinal plant widely distributed in Bangladesh and India [1]. This plant has been reported as an effective remedy for the treatment of a variety of diseases. Fruits are used as appetizing and digestive, diuretic, in jaundice and enlarged spleen. Barks are used for the treatment of intermittent fever. Roots are used in nephritic colic and gum is used in cholera [1, 2].

Previous phytochemical investigation on this plant resulted in the isolation of β-sitosterol (a well-known phytosterol), β-sitosterol-β-D-glucopyranoside, ramontoside, butyrolactone lignan disaccharide [3], and flacourtin [4]. In our previous study, we also reported the presence of coumarin such as scoparone and aesculetin [2]. Though this plant is being used in the traditional medical practice by the indigenous physicians in Bangladesh and India, but its ethnomedical values have not been evaluated through scientific screening. The present study was designed to investigate the biological effects of the plant using animal and biochemical methods. Here, we report the hepatoprotective activities of different solvent extractives of *F. indica* in paracetamol-induced hepatic necrosis in rat models.

2. Materials & Methods

2.1. Plant material

Aerial parts of *F. indica* were collected from Savar, Dhaka and taxonomically identified at the Department of Botany, University of Dhaka, where a voucher specimen has been retained. The plant was first sun dried for 3 days and then dried in an oven at 40 °C for 12 hours and ground into a coarse powder with the help of an attrition type grinder. The course powder was stored in an air-tight container and kept in a cool, dark and dry place.

2.2. Extraction

The dried powdered plant material (500 g) was extracted successively with petroleum ether (PE, 4L), ethyl acetate (EA, 3.5L) and methanol (ME, 3.5L) using maceration for 7 days in each solvent system with occasional shaking at room temperature. All the extracts were filtered through cotton bed followed by Whatman no. 1 filter paper. The filtrates were evaporated under reduced and low temperature (40 °C) using a Buchii Rotaroy Evaporator to have a gummy concentrate of the three extracts termed crude extracts. The yield of was 0.75% for petroleum extract, 0.93% for ethyl acetate extract and 1.16% for methanol extract.

2.3. Phytochemical screening of the extracts

Phytochemical investigation of the extractives were carried out to confirm the presence of different types secondary metabolites by thin layer chromatographic techniques followed by chemical detection on the TLC using different types of specific reagents [5-7]. TLC analysis revealed that petroleum ether extract showed positive test for steroids and terpenoids, ethyl acetate showed positive test for terpenoids and coumarins, and methanol extract showed positive test for terpenoids.

2.4. Animals

Long Evans rats (3-4 weeks old, 120-140 g) of either sex were used in this study. These experimental animals were obtained from the Animal House of the International Centre for Diarrheal Disease and Research, Bangladesh (ICDDR’B). Animals were maintained under standard environmental conditions and had free access to feed and water ad libitum. Experiments on animals were performed strictly in accordance
with the guidelines provided by the Institutional Animal Ethics Committee.

2.5. **Test samples and paracetamol**

Suspension of all extractives and paracetamol were prepared in normal saline using 5% acacia mucilage. All test samples and paracetamol were administered orally by feeding needle.

2.6. **Evaluation of hepato-protective activity**

Hepato-protective activities of the crude petroleum, ethyl acetate and methanol extracts were screened using paracetamol-induced hepatic necrosis rat model [8, 9]. Experimental animals were randomly selected and divided into five groups consisting of six rats in each group. Each group received a particular treatment at a single oral dose, e.g. control group received normal saline with 5% acacia mucilage. On the other hand, the test groups received PE extract (1.5 g/kg of body weight), EA extract (1.5 g/kg of body weight) and ME extract (1.5 g/kg of body weight) followed by a single oral dose of paracetamol at 3 g/kg of body weight, respectively. Hepatotoxicity was induced by oral administration of a single dose of paracetamol (3 g/kg body weight). After 72 h of treatment, blood was collected by puncturing the retro-orbital plexus and was allowed to clot at room temperature. Serum was separated by centrifugation at 3000 rpm for about 5 minutes. The clear straw colored serum was collected and stored at 4 °C for the measurement of marker enzymes levels to assess the liver functions.

2.7. **Estimation of serum enzyme levels**

Biochemical parameters such as SGOT, SGPT and SAP levels in the serum of control, paracetamol and extractives treated rats were measured to assess the protective activities of the extracts using auto-analyzer (Technocon, UK). Briefly, the substrate α-oxaloglutamate was used for the determination of SGOT and SGPT level, whereas p-nitrophenyl phosphate was used for the determination of SAP. Serum solution (100 µl) was mixed with 1 ml of each substrate solution and shaken well in a small test tube and the mixture was immediately placed in a previously programmed auto-analyzer for each marker enzymes. The values were obtained as U/L.

2.8. **Histo-pathological examination**

Livers from all experimental rats were collected and preserved in 10% formalin solution. Representative blocks of tissues from each lobe of liver were taken and then processed for paraffin impregnation. In brief, after over night fixation in formalin, the tissue blocks were dehydrated and cleared using ethanol and xylol, respectively, and then impregnated in paraffin. Microtome was used for section cutting from each lobe at 6-micron thickness. The sections were taken on glass slides previously smeared with egg albumin and then stained using haematoxylin-eosin dye, and finally mounted in diphenylxylene. The stained sections of the liver were examined under low/high beam microscope to detect the changes in hepatic texture and their photomicrographs were taken.

2.9. **Statistical analysis**

The biochemical data were analyzed by SPSS. Experimental data were expressed as mean±SEM. Independent sample t-test was carried out for statistical comparison. Percentage reductions against the paracetamol by test samples were calculated by considering enzyme level difference between the paracetamol treated and the control group as 100% levels of reduction. Statistical significance was considered to be indicated by a p value <0.001.
3. Results & Discussion

Treatment with paracetamol by administering 3 g per kg of body weight at a single dose in Long Evans rats caused considerable increase of SGOT (also known as aspartate transaminase, AST), SGPT (also known alanine transaminase, ALT) and SAP. Concomitant administration of petroleum ether and ethyl acetate extract of the aerial parts of *F. indica* with paracetamol significantly attenuated the serum concentration of SGOT and SGPT in comparison to that of paracetamol treatment and the results were found to be statistically significant (Table 1). On the other hand, methanol extract of the plant showed no remarkable effect on serum concentration of SGOT, SGPT in paracetamol induced hepatic necrosis. Moreover, none of the extracts showed any significant change in SAP level in serum.

Estimation of the serum level revealed that paracetamol induced a remarkable increase of SGOT, SGPT and SAP by 124.1%, 135.8% and 20.5%, respectively in comparison to the control animals. The mean values of SGOT in control and paracetamol treated animals were 27.90±0.83 and 62.52±0.53, respectively, whereas the mean levels of SGPT were found to be 28.82 ±0.66 and 67.95±1.04, respectively. Petroleum ether extract reduced these paracetamol induced level of SGOT and SGPT by 29.0% and 24.0%, respectively. On the other hand ethyl acetate extract reduced the concentration of SGOT and SGPT by 10.57% and 6.7%, respectively. The results were found to be statistically significant and the ‘p’ values were less than 0.001%. These observations inferred that petroleum ether and ethyl acetate extract might contain hepatoprotective principles.

Paracetamol at higher doses causes hepatic necrosis due to increased formation of reactive intermediate such as N-acetyl-p-benzo-quinone imine (NAPQI) by oxidation through the cytochrome P-450 mixed function oxidase system and NAPQI is then irreversibly conjugated with the SH groups of glutathione [9]. Attenuation of the necrotic effect of paracetamol by the extract might be due the reversible inhibition of the oxidative enzymes [10-12]. Thin layer chromatographic screening and isolation of phytochemicals from *F. indica* showed the presence of flavonoids, polyphenols and other compounds [2, 4].

**Table 1.** Effects of PE, EA and ME⁸ extracts on paracetamol-induced hepatotoxicityᵇ.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean serum concentration (U/L) ± SEM (Percent change)</th>
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<tbody>
<tr>
<td></td>
<td>SGOT</td>
</tr>
<tr>
<td>Control (saline)</td>
<td>27.90±0.83</td>
</tr>
<tr>
<td>Paracetamol (3 g/kg)</td>
<td>62.52±0.53</td>
</tr>
<tr>
<td>Paracetamol (3 g/kg) + PE (1.5 g/kg)</td>
<td>44.38±0.97*</td>
</tr>
<tr>
<td></td>
<td>(-29.0)</td>
</tr>
<tr>
<td>Paracetamol (3 g/kg) + EA (1.5 g/kg)</td>
<td>55.91±1.40*</td>
</tr>
<tr>
<td></td>
<td>(-10.57)</td>
</tr>
<tr>
<td>Paracetamol (3 g/kg) + ME (1.5 g/kg)</td>
<td>61.09±0.87</td>
</tr>
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<td></td>
<td>(-2.29)</td>
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⁸PE = Petroleum ether extract, EA = Ethyl acetate extract, ME = Methanol extract; bⁿ = 6, + Indicates increase; - indicates decrease; *p<0.001
Flavonoids and polyphenols possess antioxidative properties [13, 14]. Histo-pathological examination of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Figure 1A). In the liver sections of paracetamol intoxicated rats (Figure 1B), there was disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis, sinusoidal hemorrhages, fatty changes, vacuolization and inflammatory changes. Treatment with the extractives, especially petroleum ether extract exhibited prominent protection against paracetamol intoxication, which was evidenced by less centrilobular necrosis, less vacuole formation, reduced sinusoidal dilation, and less disarrangement and degeneration of hepatocytes (Figure 1C & 1D). These studies suggest that *F. indica* protected the hepatic necrosis by inhibiting enzymatic oxidation.

In conclusion, we infer that *F. indica* might posses some natural leads that may have antioxidative effects against drug metabolizing cytochrome P-450s. Further, investigations on this plant are to be carried out to establish the hepatoprotective potential of this plant as herbal.

**Figure 1.** Photomicrograph of liver tissue (400X). A) Control rats. B) Paracetamol treated rats (3 g/kg of body weight). C) Paracetamol and petroleum extract treated rats. D) Paracetamol and ethyl acetate extract treated rats. E) Paracetamol and methanol extract treated rats. Paracetamol intoxication caused centritubular necrosis, fatty changes and inflamed cells (B), which was reduced by petroleum ether and ethyl acetate extract of *F. indica* (C & D, respectively).
References


