Curative effects of Cassia fistula fruit extract on bromobenzene induced hepatotoxicity and nephrotoxicity in mice

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Abstract

Background & objectives: The present investigation was designed to evaluate the post-treatment properties of the hydroalcoholic fruit extract of cassia fistula (golden shower tree) in male Albino mice.

Materials & Methods: Biomarkers and histological liver and kidney alterations, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transferase (γ-GT), bilirubin, blood urea nitrogen (BUN) and creatinine were evaluated. Animals were divided into six groups: negative control (received daily normal saline), positive control (received daily bromobenzene (BB) 460 mg/kg) and groups three to six received 200, 400, 600 or 800 mg/kg hydroalcoholic C. fistula extract as oral gavages for 10 days two hours following an administration of BB 460 mg/kg.

Results: Administration of 460 mg/kg dose of BB produced a significant increase in the activities of biomarker enzymes [except for direct bilirubin (DBIL)], when compared to negative control (P<0.05). C. fistula fruit extract administration significantly decreased the activities of biomarkers to control values. The high doses (600-800 mg/kg vs. group 2) produced a significant fall in serum γ-GT activities.

Conclusions: The effects allow us to suggest that C. fistula fruit extract might represents a new therapeutic option for induced hepatotoxicity and nephrotoxicity.

Keywords: C. Fistula Linn; Bromobenzene; Hepatotoxicity; Nephrotoxicity; Mice

1. Introduction

Bromobenzene (BB) exposure may occur during release to the environment, its production and phenyl magnesium bromide production as well as in its use as a solvent and as an additive in motor oil¹, or even in table-ready foods². The metabolism and toxicity of BB in rat liver have been studied³. The metabolites of BB are highly hepatotoxic. At high doses, BB metabolites deplete the hepatic GSH pool, and may lead to several secondary events such as lipid peroxidation, local inflammation, ATP depletion, mitochondrial dysfunction, energy imbalance, and altered intracellular calcium levels⁴.

C. fistula Linn. (Family: Caesalpinaeae) is commonly called Indian Labernum or golden shower tree and is
2. Materials and Methods

Chemicals

All chemicals of inclusive methanol, ethanol, formalin, normal saline and paraffin were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Animals

Sixty adult male mice (Albino Strain) were obtained from the Animal House, School of Pharmacy Ahvaz Jundishapur University of Medical Sciences, Iran. The animals (age, 6–8 weeks) weights were about 20–28 g. Animals were randomly allocated into six groups with 10 animals in each group. Animals were housed in polycarbonate cages (60x45x15cm) and allowed to feed ad libitum. The six groups are; Group 1 (negative control group): mice received daily 0.2 ml normal saline (NS) by gavages as a vehicle. Group 2 (positive control group): received a daily single dose of BB (460 mg/kg) (6) by intra gastric intubation, for 10 days. Oral gavage of 460 mg/kg BB was administered in groups 3 to 6. Two hours later, group 3 to 6 received daily 200, 400, 600 or 800 mg/kg hydroalcoholic C. fistula extract, respectively.

The Ethic Committee of the Jundishapur University, Ahvaz approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health (NIH).

Preparation of the hydroalcoholic C. fistula extract

Three hundred grams of fruit C. fistula were collected during late autumn from a garden in the city of Ahvaz, southwest Iran. The plant was taxonomically identified at the department of botany, school of sciences, Ahvaz University. The fruits were cut into small pieces, crushed, saturated with 90% ethanol. The suspension was filtered through muslin cloth and the filtrate was rotary evaporated to dryness, using a vacuum evaporator (Adolphe, Model 462, Germany). The ethanolic C. fistula extract was viscous oily mass and the density was measured by a picrometer.

Samples

After 12 hours fasting, animals were sacrificed by decapitation and blood samples were collected and processed to obtain serum for determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (γ-GT), blood urea nitrogen (BUN), creatinine and bilirubin. The liver and kidney tissues were isolated, weighted and put in formalin (10%). Tissue samples were cut into 5µm slices, and then stained with hematoxylin-eosin (H&E×100). The blood samples were centrifuged at 300 rpm for 10 min (Hitachi model EBA 12R, Germany). The serums were stored for subsequent determinations.

Biochemical assays

Liver aspartate aminotransferase and alanine aminotransferase activities were assayed by the Reitman and Frankel method22. The total bilirubin was determined according to the Jendressik- Grof method23. Alkaline phosphatase (ALP) was determined as described previously by King24. γ-Glutamyl transferase (γ-GT) was determined previously by Rosalki and Rai25. Blood urea nitrogen (BUN) was determined according to method of Zimmerman25. Creatinine was determined according to the method of Huseby and Ingebritsen22.

Statistical analysis

All data are presented as mean ± SEM. All analyses were carried out using SPSS software. Student’s t-test and ANOVA were used to assess differences. Probability values <0.05 were considered to be statistically significant.
3. Results

The protective effect on the serum biomarkers of liver injury

The activity of AST, ALT, ALP, BIL and δ-GT are presented in Table 1. It is observed that administration of 460 mg/kg dose of BB (Group -II) produced a significant increase in the activities of biomarker enzymes [except for direct bilirubin (DBIL)], when compared to group 1 (negative control) (P<0.05). *C. fistula* fruit extract administration (Group -III to VI) significantly decreased the activities of biomarkers to control values. The extract produced a significant fall in the levels of AST, ALT, ALP, BIL (liver toxicity markers) in a dose-dependent manner (groups 3-6 vs. group 1 (control); (Table 1).

Higher doses (600-800 mg/kg vs. group 2) produced a significant fall in serum δ-GT activities. The fall was not significant at lower doses (200 and 400mg/kg) (Table 1).

Table 1: Effect of BB and *C. fistula* fruit extract on the status of serum biomarkers of liver injury, DBIL (direct bilirubin), TBIL (total bilirubin), INBIL (indirect bilirubin), AST, ALT and ALP of the experimental animals. Results are given as mean ± SEM, for 10 mice. Units: -GT, AST, ALT in IU/L and BIL, DBIL, INBIL in mg/dL.

<table>
<thead>
<tr>
<th>Group</th>
<th>DBIL</th>
<th>TBIL</th>
<th>DBIL/TBIL</th>
<th>INBIL</th>
<th>AST</th>
<th>ALT</th>
<th>ALK</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>0.228±0.018</td>
<td>0.557±0.02</td>
<td>0.409</td>
<td>0.385±0.055</td>
<td>243.14±12.66</td>
<td>75±6.07</td>
<td>170.66±4.55</td>
</tr>
<tr>
<td>BB</td>
<td>0.233±0.021</td>
<td>0.716±0.047*</td>
<td>0.325</td>
<td>0.5±0.044*</td>
<td>320.33±13.37*</td>
<td>122.5±2.14*</td>
<td>326.33±10.03*</td>
</tr>
<tr>
<td>200</td>
<td>0.233±0.021</td>
<td>0.633±0.066</td>
<td>0.368</td>
<td>0.333±0.049*</td>
<td>278.16±21.29*</td>
<td>113±14.67*</td>
<td>230.16±17.13*</td>
</tr>
<tr>
<td>400</td>
<td>0.22±0.02</td>
<td>0.64±0.05</td>
<td>0.343</td>
<td>0.4±0.044*</td>
<td>264±6.27*</td>
<td>108±25.54*</td>
<td>210±10.07*</td>
</tr>
<tr>
<td>600</td>
<td>0.233±0.021</td>
<td>0.56±0.033*</td>
<td>0.411</td>
<td>0.366±0.049*</td>
<td>258±14.38*</td>
<td>91±6.76*</td>
<td>192.66±14.23*</td>
</tr>
<tr>
<td>800</td>
<td>0.225±0.025</td>
<td>0.55±0.064*</td>
<td>0.409</td>
<td>0.325±0.047*</td>
<td>248±19.12*</td>
<td>89.5±9.53*</td>
<td>190.5±12.81*</td>
</tr>
</tbody>
</table>

Comparisons are made between: a, Negative control; b, BB treated mice. The symbol (*) represent statistical significance at P<0.05.

The protective effect on the serum biomarkers of renal injury

The activity BUN and creatinine in the control and experimental groups are presented in Table 2. Treatment with *C. fistula* fruit extract significantly decreased the activities of BUN and creatinine in a dose-dependent manner.

Treatment significantly decreased the activities of serum BUN in a dose-dependent manner [groups 4-6 (400-600-800 mg/kg) vs. group 2; Table 2]. The low dose (200 mg/kg) did not exhibit a significant fall when compared with control values (group 3) (200mg/kg) vs. group 1 (control) (Table 2).

Table 2: Effect of BB and *C. fistula* fruit extract on the status of serum biomarkers of renal injury, BUN and creatinine (Cr) of the experimental animals. Results are given as mean ±S.E. for 10 mice. Units: BUN and creatinine in mg/dL.

<table>
<thead>
<tr>
<th>Group</th>
<th>BUN</th>
<th>Cr</th>
<th>BUN/ Cr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>18.587±2.472</td>
<td>0.267±0.032</td>
<td>72/32</td>
</tr>
<tr>
<td>BB</td>
<td>29.245±1.36*</td>
<td>0.411±0.038*</td>
<td>68/81</td>
</tr>
<tr>
<td>200</td>
<td>23.5±3.5</td>
<td>0.346±0.044</td>
<td>67/9</td>
</tr>
<tr>
<td>400</td>
<td>22±1.048*</td>
<td>0.33±0.02*</td>
<td>66/6</td>
</tr>
<tr>
<td>600</td>
<td>21±2.56*</td>
<td>0.31±0.026*</td>
<td>67/7</td>
</tr>
<tr>
<td>800</td>
<td>20±0.816*</td>
<td>0.26±0.06*</td>
<td>76/9</td>
</tr>
</tbody>
</table>

Comparisons are made between: a, negative control mice; b, BB treated mice. The symbol (*) represent the statistical significance at P<0.05.

Histological results

Histological profile of renal samples received BB alone showed a necrotic lesion (Fig 1B for liver, Fig 2B for kidney). The post-treatment effects of *C. fistula* fruit extract in different dose (200, 400, 600 and 800mg/kg) were confirmed by histopathological examination of the liver and kidney sections of control (A), liquid paraffin: BB (B) and the extract treated groups (Fig 1C-F for liver, Fig
Administration of the extract to experimental animals at four doses (200, 400, 600 and 800 mg/kg) exhibited a significant improvement of hepatocellular architecture. This effect is evident from a considerable reduction in necrosis and fatty changes (Fig 1C-F vs. B). Administration of the extract in four doses showed a significant improvement in the architecture of the kidney. This effect is evident from the reduction of necrosis (Fig 2C-F vs. B).

Figure 1. Light microscopic photos of liver section from mice. Hematoxylin and Eosin (H&E×100): (A) given normal saline (NS), (B) given Bromobeneze (BB) 460 mg/kg, (C) given BB and *C. fistula* fruit 200 mg/kg, (D) given BB and *C. fistula* fruit 400 mg/kg, (E) given BB and *C. fistula* fruit 600 mg/kg, (F) given BB and *C. fistula* fruit 800 mg/kg. Administration of the extract exhibited a significant improvement of hepatocellular architecture in a dose-dependent manner.

Figure 2: Light microscopic photos of kidney section from mice. Hematoxylin and Eosin (H&E×100): (A) given normal saline (NS), (B) given Bromobeneze 460 mg/kg (BB), (C) given BB and *C. fistula* fruit 200 mg/kg, (D) given BB and *C. fistula* fruit 400 mg/kg, (E) given BB and *C. fistula* fruit 600 mg/kg, (F) given BB and *C. fistula* fruit 800 mg/kg. Administration of the extract showed a significant improvement in the architecture of the kidney indicated by the reduction of necrosis.

4. Discussion

The results suggest that *Cassia fistula* fruit extract has hepato- and nephrocurative effects against bromobenzene (BB) induced hepatotoxicity or nephrotoxicity in mice. We have previously shown that *C. fistula* fruit extract has the protective effects on bromobenzene (BB) induced hepatotoxicity or
nephrotoxicity in mice. Histopathological profiles of the liver and kidney sections following post-treatment administration showed a significant improvement in induced intense centrilobular necrosis, stenosis and swelling of hepatic cytoplasm by BB administration. The results showed the curative effects of *C. fistula* fruit on BB induced hepatotoxicity and nephrotoxicity by normalization of the biomarkers AST, ALT, ALP, γGT, BUN, creatinine and liver function test, bilirubin. 

High serum concentrations of transaminases are taken as an index of hepatic injury. Elevation of ALT is regarded as a more sensitive indicator. These markers are also known to be distinct in rodents. Elevated activities of transaminases in serum might be due to the release of these enzymes from the cytoplasm into the blood circulation immediately after rupture of the plasma membrane and cellular damage. Free radicals released by the metabolism of BB might cause damage to the hepatocellular membranes and kidney proximal tubuli. These radicals cause liver toxicity either directly from the disruption of intracellular function or membrane integrity or even from damage affecting endothelial or bile duct cells.

Changes in serum bilirubin (total and direct values) have a similar pattern with previous results (pretreatment administration). Bilirubin is eliminated by the liver via hepatocellular uptake, conjugation (a phase II reaction) and secretion into the bile. While total bilirubin refers to both conjugated and non-conjugated bilirubin, damage of liver function leads to increased bilirubin concentrations in serum. In the current study both direct and total bilirubin levels were elevated in positive group (BB alone administration). Slight decrease in the direct-to-total bilirubin ratio showed a predominance of non-conjugated bilirubin. Post-treatment showed reversing the damage in hepatic conjugation and biliary excretion of bilirubin in a dose-dependent manner. These reversing were confirmed by the recovery of direct and total bilirubin concentrations as well as the direct-to-total bilirubin ratio (especially in 800 mg/kg dose).

γ-GT is considered to be a more sensitive indicator of hepatobiliary disease. Therefore measurement of serum γ-GT is a frequently used parameter in liver diseases. The elevated activity of γ-GT may be due to induced hepatic necrosis or hepatocellular lesions by bromobenzene. Post-treatment with high dose of *C. fistula* fruit extract significantly reduced the levels of these biomarkers. This effect shows that *C. fistula* fruit extract stops liver damage by suppressing the leakage of biomarkers by preserving the integrity of the plasma membranes and restoring the status of this enzyme.

The 10-day oral BB administration caused renal injury. This effect was shown by the elevation of BUN and serum creatinine levels, the two most important laboratory parameters of renal function.

In the present study, we were able to confirm and provide more evidences for the efficacy of *C. fistula* fruit to the BB-induced nephrotoxicity. Post-treatment like pre-treatment administration, reduced the levels of both serum BUN and creatinine. This effect was especially showed at the highest dose, 800 mg/kg (Table 2). The BUN-to-creatine ratio was reversed to normal level in a dose-dependent manner. Thus as previously suggested and discussed, it can be concluded that renal tubular function is more sensitive to the nephrotoxic agents including BB than the glomerular filtration. Histological evidences against BB-induced nephrotoxicity were shown (Fig 2). Post-treatment showed capable to reverse the renal damages in a dose-dependent manner. These effects were evidenced by the recovery of BUN and creatinine as well as of the BUN-to-creatine ratio.

Studies have already reported the ameliorative effect of leaf extract of *C. fistula* Linn, against CCl4 induced hepatotoxicity in rats, but the effectiveness of its fruit against BB induced hepatotoxicity and nephrotoxicity in mice is reported here for the first time. The concern how various constitutes of *C. fistula* fruit extract influences the biologic activity *in vivo* is the subject of further investigation. The combined results from the present study with previous studies clearly demonstrate that *C. fistula* is an important medicinal plant possessing high biologic activity against hepatotoxicity and nephrotoxicity. Based on our studies we can suggest that *C. fistula* able to provide important health benefits to humans.

Since *C. fistula* has effective antioxidant can be hypothesized that available antioxidants in the *C. fistula* fruit scavenged and detoxified the produced reactive metabolite of BB, thereby inhibiting lipid peroxidation and improving the activities of hepatotoxic and nephrotoxic biomarkers.

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References


