1 Introduction

Drug-induced nephrotoxicity is a common side effect of many therapeutic drugs. Cases of acute renal injury have been increasingly reported over the last two decades and cause morbidity and mortality among patients. Drugs such as aminoglycoside antibiotics, amphotericin B, non-steroidal anti-inflammatory drugs, cisplatin, certain cyclo-oxygenase-2 inhibitors and angiotensin converting enzyme inhibitors have been reported to cause nephrotoxicity. Other drugs like rifampicin, isoniazid, antimalarials, antivirals as well as agents like penicillin,
Bromobenzene (BB; C₆H₅-Br; molecular weight 157.02) is an xenobiotic, which is used as an additive in motor oil, as a solvent for crystallizations on a large scale and also in the production of phenyl magnesium bromide. It is a heavy, mobile, colorless liquid with a pungent odor. Absorption by way of ingestion (through contaminated food or environmental exposure) or dermal exposure is followed by the metabolism of BB in the liver, leading to hepatotoxicity. Furthermore, the secondary metabolites of BB are found to be highly toxic to the kidneys. The reactive oxygen species (ROS) generated during breakdown of BB mediates its nephrotoxic effect, leading to renal necrosis and tubular degeneration. Studies in experimental rats have shown that agents with antioxidant property ameliorate the toxic effects of drug-induced nephrotoxicity. Because the side effects associated with many synthetic drugs can be as dangerous and serious as the ailments they claim to cure, there has been a return to natural remedies to treat various clinical conditions.

Triphala, comprising three fruits, Terminalia bellerica, Terminalia chebula and Emblica officinalis, is one of the most common herbal formulations used in Indian Ayurvedic medicine. It is believed to promote health, render immunity and rejuvenate. The antioxidant-rich herbal formulation is used in the treatment of several clinical conditions such as jaundice, asthma, constipation, fever, fatigue, anemia, vomiting, typhoid, chronic ulcers, obesity, eye diseases, as well as in the treatment of infectious diseases such as tuberculosis, pneumonia and acquired immune deficiency syndrome. Triphala and its components have diverse medicinal properties and have been shown to possess antibacterial, antifungal, antimalarial, antiviral, anticancer, anti-inflammatory, antioxidant, hepatoprotective and gastroprotective activity. Triphala has also shown radioprotective effect in γ-radiation-induced mice. The present study was undertaken to assess the protective effect of Triphala in BB-induced nephrotoxicity in rats. The standard drug silymarin was used as a reference drug for the purpose of comparison.

2 Materials and methods

2.1 Drugs and chemicals

Commercially available Triphala powder was obtained two weeks before the commencement of the experiment from the Indian Medical Practitioners Co-operative Stores and Society (IMCOPS; Mylapore, Chennai, India). Silymarin capsules were purchased locally. BB was purchased from Sigma Chemical Co. BB was dissolved in coconut oil while silymarin and Triphala were dissolved in sterile distilled water. Commercial reagent kits (AutoSpan Diagnostics, India) were used for the estimation of serum creatinine, urea, uric acid, albumin and total protein levels. All other chemicals and reagents used were of analytical grade and purchased locally.

2.2 Animals

The study was carried out using female Wistar albino rats with a mean weight of (153±13) g (Animal House, VIT University, Vellore, Tamil Nadu, India). The animals were housed 6 per cage in a light- and temperature-controlled room (24±2 °C; 12-hour dark-light cycles). The animals were acclimatized for a week before the study began, and were allowed access ad libitum to standard pelleted feed (Hindustan lever Ltd., Mumbai, India) and water. Guidelines recommended by the Committee for the Purpose of Supervision and Control of Experiments on Animals (CPSCEA), Government of India, were followed for the care and maintenance of the animals. The experimental procedure was approved (IAEC/VIT/VIII/8) by the Ethical Committee of VIT University, Vellore, India.

2.3 Experimental design

Animals were divided into five groups of six rats and received experimental treatment for 8 d. Rats in Group I, the normal control, received 1.5 mL of coconut oil on day 1 and normal food and water after that until the end of the experiment. In Group II, toxicity was induced with a single oral dose of BB (10 mmol/kg) on day 1 after which normal food and water was given until day 8. In Group III, a single oral dose of BB (10 mmol/kg) was given on day 1 followed by a daily dosage of Triphala (250 mg/kg). Rats in Group IV received a single oral dose of BB (10 mmol/kg) on day 1 followed by a daily dosage of Triphala (500 mg/kg). Rats in Group V received no BB, and a daily dosage of Triphala (500 mg/kg). Finally, animals in Group VI received a single oral dose of BB (10 mmol/kg) on day 1 followed by a daily dosage of the standard drug silymarin (100 mg/kg).

The oral administration of Triphala or silymarin continued until day 8 and the animals were sacrificed 18 h after the last dosage under ether anesthesia. Trunk blood was collected and the kidneys were isolated for further analysis. Blood samples were centrifuged at 252 × g for 10 min to obtain serum. The kidney tissues were washed and homogenized (100 mg in 10 mL buffer) in 0.1 mol/L phosphate-buffered saline (PBS). The homogenates were centrifuged at 252 × g for 15 min and the resulting supernatants were separated and used for biochemical assays.
2.4 Histopathological examination

Immediately after sacrifice, a portion of the kidney from each experimental rat was removed separately in 10% formalin. These tissues were processed, dehydrated in ascending grades of alcohol, and stained with haematoxylin and eosin (HE) and examined microscopically for histopathological changes.

2.5 Biochemical parameters

The levels of total protein, albumin, creatinine, urea and uric acid were determined in serum using diagnostic kits according to the protocol given by the manufacturers. Assays of antioxidant enzymes like catalase (CAT)\(^{[22]}\), superoxide dismutase (SOD)\(^{[23]}\), glutathione-S-transferase (GST)\(^{[24]}\), glutathione peroxidase (GSH-Px)\(^{[25]}\), total reduced glutathione (GSH)\(^{[26]}\) as well as the lipid peroxidation levels\(^{[27]}\) were determined in the kidney tissue homogenates. Total protein was estimated using bovine serum albumin as standard\(^{[28]}\). Acid phosphatase was estimated in the serum of the experimental rats by the method of King\(^{[29]}\).

2.6 Statistical analysis

The experimental data are represented in the text as mean ± standard deviation. Comparisons among treatment groups were made using one-way analysis of variance (ANOVA), followed by the Student-Newman-Kuels (SNK) test. For all comparisons the significance level of \(P<0.05\) was used.

### Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Reduced glutathione (nmol/mg protein)</th>
<th>Lipid peroxidation (nmol of MDA formed/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (control)</td>
<td>6</td>
<td>46.63±1.20</td>
<td>1.20±0.06</td>
</tr>
<tr>
<td>Group II (BB 10 mmol/kg)</td>
<td>6</td>
<td>29.49±0.84*</td>
<td>2.59±0.15*</td>
</tr>
<tr>
<td>Group III (BB + Triphala 250 mg/kg)</td>
<td>6</td>
<td>41.67±1.13*</td>
<td>1.49±0.08*</td>
</tr>
<tr>
<td>Group IV (BB + Triphala 500 mg/kg)</td>
<td>6</td>
<td>42.63±1.21*</td>
<td>1.48±0.09*</td>
</tr>
<tr>
<td>Group V (Triphala 500 mg/kg)</td>
<td>6</td>
<td>47.16±1.41 △</td>
<td>1.16±0.05 △</td>
</tr>
<tr>
<td>Group VI (BB + silymarin 100 mg/kg)</td>
<td>6</td>
<td>41.91±1.07*</td>
<td>1.50±0.09*</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± standard deviation. \(^* P<0.05\), vs Group I; \(^\triangle P<0.05\), vs Group II; \(^\ddagger P<0.05\), vs Group III; \(^\square P<0.05\), vs Group IV; \(^\bullet P<0.05\), vs Group V. BB: bromobenzene; MDA: malondialdehyde.

### Table 2

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Catalase (µmol of H(_2)O(_2) consumed/ min/mg protein)</th>
<th>Glutathione peroxidase (µg of GSH utilized/min/mg protein)</th>
<th>Superoxide dismutase (U/mg protein)</th>
<th>Glutathione-S-transferase (nmol of CDNB-GSH conjugate formed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (control)</td>
<td>6</td>
<td>11.93±0.66</td>
<td>65.60±1.40</td>
<td>218.75±2.68</td>
<td>87.38±1.41</td>
</tr>
<tr>
<td>Group II (BB 10 mmol/kg)</td>
<td>6</td>
<td>6.95±0.57(^*)</td>
<td>35.28±0.98(^*)</td>
<td>111.97±2.13</td>
<td>58.91±1.27</td>
</tr>
<tr>
<td>Group III (BB + Triphala 250 mg/kg)</td>
<td>6</td>
<td>9.96±0.63(^*)</td>
<td>60.61±1.08(^*)</td>
<td>206.78±2.10(^*)</td>
<td>81.94±1.04(^*)</td>
</tr>
<tr>
<td>Group IV (BB + Triphala 500 mg/kg)</td>
<td>6</td>
<td>10.69±0.54(^*)</td>
<td>62.12±1.08(^*)</td>
<td>212.90±2.19(^*)</td>
<td>84.32±1.24(^*)</td>
</tr>
<tr>
<td>Group V (Triphala 500 mg/kg)</td>
<td>6</td>
<td>11.78±0.71(^*)</td>
<td>67.08±1.43(^*)</td>
<td>216.86±2.71(^*)</td>
<td>88.67±1.33(^*)</td>
</tr>
<tr>
<td>Group VI (BB + silymarin 100 mg/kg)</td>
<td>6</td>
<td>10.16±0.76(^*)</td>
<td>62.05±1.13(^*)</td>
<td>210.29±1.94(^*)</td>
<td>85.97±1.13(^*)</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± standard deviation. \(^* P<0.05\), vs Group I; \(^\triangle P<0.05\), vs Group II; \(^\ddagger P<0.05\), vs Group III; \(^\square P<0.05\), vs Group IV; \(^\bullet P<0.05\), vs Group V. BB: bromobenzene; GSH: glutathione; CDNB: chlorodinitrobenzene.
significantly different between the group receiving Triphala alone (Group V) and the normal control (Group I).

3.2 Renal function markers

BB-treated rats (Group II) showed significantly ($P < 0.05$) higher levels of serum creatinine, urea and uric acid when compared to the normal control group (Group I, Table 3). Group II rats also had a significant ($P < 0.05$) increase in the level of acid phosphatase relative to the Group I animals. Triphala treatment significantly ($P < 0.05$) reduced the high levels of creatinine, urea and uric acid relative to Group II and reversed the nephrotoxicity induced by BB. In addition, Triphala treatment decreased the elevated acid phosphatase levels relative to the Group II. At the higher dose of Triphala (Group IV), urea, creatinine, uric acid, total protein, albumin and acid phosphatase levels were all indistinguishable from the normal control. These effects were not significantly different compared with those of the standard drug silymarin. In the group receiving Triphala alone (Group V), the tested serum parameters were not significantly different from the normal control (Group I).

3.3 Kidney histology

Normal glomerular and tubular histology was seen in the normal control rats (Group I), while the BB-treated rats (Group II) showed severe renal tubular damage and necrosis with extensive and marked glomerular widening (Figure 1B). In contrast, Triphala (250 mg/kg) treatment in the Group III rats reduced the renal damage caused by BB and resulting in only mild tubular damage (Figure 1C). The higher dose of Triphala (500 mg/kg) given to the Group IV rats rescued the renal toxicity of BB and restored normal kidney histology and normal glomerular architecture (Figure 1D). Group V rats, receiving Triphala alone showed normal kidney histology (Figure 1E). The silymarin-treated group (Group VI) showed nearly normal kidney architecture (Figure 1F).

4 Discussion

The bio-activation of xenobiotics, most often associated with their detoxification, makes them highly toxic to the liver and other extra-hepatic tissues, of which the kidneys are the most affected[30]. It is known that xenobiotics can induce phases I, II and III drug metabolism and transport. It follows that, the levels of the phases I and II drug-metabolizing enzymes (DMEs) and the phase III transporters are found to be elevated after exposure to xenobiotics[31,32]. Phase I DMEs consist primarily of the cytochrome P450 superfamily while the phase II DMEs include mainly the family of sulfotransferases, GST, uridine diphosphate-glucuronosyltransferases and N-acetyltransferases[33]. The activity of these enzymes on BB leads to the generation of reactive metabolites that cause depletion of GSH and thus result in reduced antioxidant status as evidenced by significant decreases in the levels of the enzyme antioxidants (CAT, SOD, GST and GPx) and reduced GSH which were in accordance with previous studies[34].

Lipid solubility facilitates gastrointestinal absorption and BB being a lipophilic xenobiotic it is poorly excreted by the kidneys[35]. Thus, the secondary metabolites of BB in conjunction with glutathione generate toxic derivatives that accumulate in the kidneys, causing tubular damage and necrosis[8]. In the present study, a similar pattern was observed, and significantly elevated levels of the serum markers for renal dysfunction were found in BB-treated rats. Creatinine is one classic marker of renal dysfunction. Urea and uric acid are also important parameters in the clinical evaluation of renal impairment. The reduced renal clearance of creatinine, urea and uric acid indicates nephropathy in rats treated with BB. Treatment with Triphala restored normal glomerular filtration and renal function, and normalized these markers. Triphala treat-

### Table 3 Effects of Triphala on the levels of kidney functional markers in the serum of BB-intoxicated rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>Uric acid (mg/dL)</th>
<th>Total protein (g/dL)</th>
<th>Albumin (g/dL)</th>
<th>Acid phosphatase (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (control)</td>
<td>6</td>
<td>10.41±0.93</td>
<td>0.62±0.07</td>
<td>6.60±0.56</td>
<td>6.73±0.46</td>
<td>3.92±0.18</td>
<td>0.14±0.01</td>
</tr>
<tr>
<td>Group II (BB 10 mmol/kg)</td>
<td>6</td>
<td>40.85±1.99</td>
<td>2.92±0.21</td>
<td>21.47±1.02</td>
<td>4.03±0.39</td>
<td>2.67±0.11</td>
<td>0.34±0.02</td>
</tr>
<tr>
<td>Group III (BB + Triphala 250 mg/kg)</td>
<td>6</td>
<td>12.86±1.04</td>
<td>0.81±0.10</td>
<td>8.07±0.69</td>
<td>6.12±0.48</td>
<td>3.64±0.17</td>
<td>0.17±0.01</td>
</tr>
<tr>
<td>Group IV (BB + Triphala 500 mg/kg)</td>
<td>6</td>
<td>11.05±1.09</td>
<td>0.75±0.09</td>
<td>7.23±0.65</td>
<td>6.68±0.51</td>
<td>3.70±0.18</td>
<td>0.16±0.01</td>
</tr>
<tr>
<td>Group V (Triphala 500 mg/kg)</td>
<td>6</td>
<td>9.90±0.87</td>
<td>0.79±0.09</td>
<td>6.20±0.48</td>
<td>7.18±0.54</td>
<td>4.05±0.21</td>
<td>0.15±0.01</td>
</tr>
<tr>
<td>Group VI (BB + silymarin 100 mg/kg)</td>
<td>6</td>
<td>11.57±0.90</td>
<td>0.60±0.08</td>
<td>7.19±0.66</td>
<td>7.19±0.66</td>
<td>3.82±0.14</td>
<td>0.19±0.01</td>
</tr>
</tbody>
</table>

The values are expressed as mean ± standard deviation. * $P < 0.05$, vs Group I; ▲ $P < 0.05$, vs Group II; △ $P < 0.05$, vs Group III; □ $P < 0.05$, vs Group IV; ■ $P < 0.05$, vs Group V. BB: bromobenzene.
ment in BB-treated rats also reduces the elevated acid phosphatase and total cholesterol to normal levels.

BB causes tubular damage and renal necrosis due to increased lipid peroxidation and the generation of its reactive metabolites, and ROS. In contrast, the oral administration of Triphala in BB-treated rats was associated with significant reduction in injury. Kidney histology of BB-treated rats receiving Triphala was barely distinguishable from that of the healthy control rats. This could be due to the rich antioxidant content of Triphala, which is known to contain active components like gallic acid, ellagic acid, chebulinic acid, phylembic acid, tannins and flavonoids\(^{21}\). These components possess antioxidant properties, which aid in stabilizing the cellular membrane by reducing the levels of lipid peroxidation, thus resulting in reduced renal cellular damage. Triphala treatment has also helped in restoring the levels of reduced GSH and antioxidant enzymes. The protective effect of \textit{T. bellerica}, one of the components of Triphala, against carbon tetrachloride-induced liver and kidney damage in albino rats was studied by Jadon \textit{et al}\(^{36}\). Another study showed the effect of the aqueous extract of \textit{T. chebula} on oxidative stress and antioxidant status in the liver and kidney of young and aged rats\(^{37}\). Also, in a randomized control trial involving obese subjects, the nephroprotective activity of Triphala was demonstrated in humans\(^{38}\). These studies are supported by our results, as they are not different from previously published data.

5 Conclusion

The present study shows that BB treatment in albino Wistar rats results in significant reduction in total protein, albumin and the antioxidant status of the animals; it also causes elevation of serum urea, creatinine, uric acid and acid phosphatase. These parameters were reduced to normal or near normal when rats received treatment with Triphala. Histological analysis of kidney tissues revealed the nephroprotective effect of Triphala in BB-intoxicated rats. Our results suggest that the amelioration of BB-induced nephrotoxicity by Triphala may be related to its antioxidant property and therefore represents a potential therapeutic strategy for renal injury caused by BB. Several clinically used therapeutic drugs like cisplatin and gentamicin are known to cause nephrotoxicity, which is similar to that of BB. Triphala may be helpful in mitigating this particular side effect of such drugs. However, the effectiveness of the active components of Triphala in acute renal failure and the mechanisms involved require further investigation.

6 Competing interests

The authors declare that they have no conflict of interests.
REFERENCES


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**Submission Guide**

*Journal of Integrative Medicine* (JIM) is an international, peer-reviewed, PubMed-indexed journal, publishing papers on all aspects of integrative medicine, such as acupuncture and traditional Chinese medicine, Ayurvedic medicine, herbal medicine, homeopathy, nutrition, chiropractic, mind-body medicine, Taichi, Qigong, meditation, and any other modalities of complementary and alternative medicine (CAM). Article types include reviews, systematic reviews and meta-analyses, randomized controlled and pragmatic trials, translational and patient-centered effectiveness outcome studies, case series and reports, clinical trial protocols, preclinical and basic science studies, papers on methodology and CAM history or education, editorials, global views, commentaries, short communications, book reviews, conference proceedings, and letters to the editor.

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