Full Length Research Paper

Alteration of renal function by potassium bromate (KBrO₃): Protective effects of Launaea procumbens

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Potassium bromate (KBrO₃) is a nephrotoxin which causes renal oxidative stress and cancer in experimental animal. In the present study, nephroprotective effects of Launaea procumbens against KBrO₃ induced renal function toxicity were determined. In this study, 24 male albino rats (180 to 200 g) were divided into 4 groups. Group I was given saline (1 ml/kg b.w., 0.85% NaCl) and dimethyl sulfoxide (DMSO) (1 ml/kg b.w.), group II was treated with KBrO₃ (20 mg/kg b.w., i.p.), groups III and IV were administered with KBrO₃ and after 48 h with methanolic fractions of L. procumbens (LPME) (100; 200 mg/kg b.w., respectively). All the treatments were given twice a week for 4 weeks. The results revealed that KBrO₃ induced oxidative stress as evidenced by the significant alteration in physical and serum markers of kidney. L. procumbens co-administration dose dependently reversed the physical and serum markers nearly to control groups. The results revealed that LPME can be a useful remedy for renal disorders.

Key words: Potassium bromate (KBrO₃), Launaea procumbens, physical analysis, white blood cells (WBCs).

INTRODUCTION

For thousands of years, medicinal plants are being used as a source of medicine and for improvement of human health. Several herbs possess bioactive constituent such as phenolic and polyphenolic compounds which regulates various immunological pathways. The flavonoids and phenolic compounds rich herbs may also possess antioxidant and anti-inflammatory properties (Tyler, 1994; Bruneton, 1995). Launaea procumbens (Roxb.) Amin. (Asteraceae) is an annual herb having simple leaves and yellow flowers found in waste places, vacant lots and in cultivated fields throughout Pakistan. L. procumbens is used as a food and washing agent (Wazir et al., 2007), rheumatism, galactogogues and increases milk production (Parikh and Chanda, 2006). Eye redness and itchiness are treated with L. procumbens (Yousaf et al., 2004) and also traditionally used in kidney (painful urination), liver and sexual diseases like gonorrhea (Ahmad et al., 2006). The antimicrobial activity of ethanolic and aqueous fractions of L. procumbens was checked against various bacterial and fungal pathogens and reported that ethanolic extract showed maximum activity while the aqueous extract was inactive (Parikh and Chanda, 2006). L. procumbens is generally classified as Kingdom (Plantae), Order (Asterales), Family (Asteraceae), Tribe (Cichorieae), Genus (Launaea), and Species (procumbens). Chemical characterization showed that L. procumbens are composed of salicylic acid, vanillic acid, syringic acid, 2-methyl-resercinol, gallic acid and used against plant pathogenic fungi, nematocides and as allelopathic for inhibition of plant growth (Shaukat et al., 2003; Khan et al., 2010a, 2010b). Present study is designed to check the protective effect of L. procumbens on urine function of kidney damaged

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induced by potassium bromate (KBrO₃).

**MATERIALS AND METHODS**

**Plant collection and extract preparation**

*L. procumbens* at maturity was collected from Bannu (Pakistan), identified and a specimen was submitted at Herbarium of Pakistan, Quaid-i-Azam University Islamabad, Pakistan. Arial parts of the plant were shade dried for 2 weeks, chopped and grinded mechanically. Two (2) kg powder of *L. procumbens* was extracted in 80% methanol (four litres) to get crude methanolic extract (LME) with refluxing for 5 h. This extract was cooled at room temperature, filtered and evaporated under reduced pressure in rotary evaporator and stored at 4ºC for further *in vivo* investigations.

**Experimental design**

Twenty-four (24) male Sprague Dawley rats (195 to 200 g) were procured from National Institute of Health, Islamabad and were kept in ordinary cages at room temperature of 25±3ºC with a 12 h dark/light cycle and allowed access to standard feed and water according to the study protocol approved by Ethical committee of Quaid-i-Azam University, Islamabad.

Animals were divided into 4 groups of 6 animals each. Group I treated with vehicle (saline) was kept as normal, while Group II received 20 mg/kg b.w KBrO₃ in saline and was kept as toxin control. Groups III and IV were treated with LME at two different doses of 100 and 200 mg/kg b.w intragastrically, respectively after 48 h of KBrO₃ treatment.

All these treatments were given for 4 weeks. After completion of treatment period, animals were kept individually in metabolic cages for 24 h to collect their urine for estimation of renal function tests. Blood was collected through cardiac puncture for serum analysis.

**Assessment of urine and serum parameters**

**Urine analysis**

Urine samples were assayed for pH, specific gravity, urea, urinary creatinine, urinary creatinine clearance, urinary protein, albumin, urobilinogen, red blood cells (RBCs) and white blood cells (WBCs) count by using standard diagnostic kits (MediScreen Urine Strips, Organics, France) and standard AMP diagnostic kits (Stattogether Strasse 31b 8045 Graz, Austria). However, pH of urine was also confirmed by using pH meter.

**Serum analysis**

Analysis of serum includes blood urea nitrogen (BUN), serum nitrite, serum creatinine, serum creatinine clearance, serum total protein, globulin and albumin. These analyses were done by using standard AMP diagnostic kits (Stattogether Strasse 31b 8045 Graz, Austria).

**Statistical analysis**

To determine the treatment effects, one-way analysis of variance (ANOVA) was carried out by computer software SPSS 13.0. Level of significance among the various treatments was determined by least square difference (LSD) at 0.05% level of probability.

**RESULTS**

**Effect of L. procumbens on physical parameters of kidney in rat**

Table 1 shows the effect of *L. procumbens* on physical parameters of kidney in rat. Administration of nephrotoxic KBrO₃ treatment significantly (*P<0.01*) augmented the level of specific gravity, urea and creatinine, while it decreased (*P<0.01*) the urinary pH and creatinine clearance than control. *L. procumbens* attenuated the KBrO₃ intoxication, and abridged (*P<0.01*) the specific gravity, urea and creatinine while it augmented the pH and creatinine clearance of urine.

**Effect of L. procumbens on urinary proteins and blood cells in rat kidney**

Effect of *L. procumbens* against KBrO₃ induced toxicity on urinary protein, albumin, urobilinogen, RBCs and WBCs level in rat are shown in Table 2. Treatment of KBrO₃ significantly increased (*P<0.01*) the level of urinary protein, urobilinogen, RBCs, WBCs and albumin concentration (*P<0.01*) in urine when compared to non-treated control group. Co-treatment of *L. procumbens* significantly changed (*P<0.01*) the toxicity level of KBrO₃ and the urinary level of these parameters returned towards the control group.

**Effect of L. procumbens on renal serum markers in rat**

Effects of *L. procumbens* renal serum markers are shown in Table 3. KBrO₃ treatment to rats significantly (*P<0.01*)...
Table 2. Effect of methanolic fraction of *L. procumbens* on urea, RBC, WBC, protein, albumin, urobilinogen in rat.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Albumin (mg/dl)</th>
<th>RBC/µl</th>
<th>WBC/µl</th>
<th>Urobilinogen (mg/dl)</th>
<th>Total protein (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>27.50 ± 1.41**</td>
<td>0.00 ± 0.00**</td>
<td>17.07 ± 1.2**</td>
<td>5.17 ± 1.26**</td>
<td>24.217 ± 0.94**</td>
</tr>
<tr>
<td>II</td>
<td>20 mg/kg KBrO₃</td>
<td>67.33 ± 4.46**</td>
<td>19.33 ± 1.39**</td>
<td>25.0 ± 1.39**</td>
<td>35.74 ± 1.18**</td>
<td>61.0 ± 1.18**</td>
</tr>
<tr>
<td>III</td>
<td>100 mg/kg LPME + KBrO₃</td>
<td>39.67 ± 2.50**</td>
<td>6.7 ± 0.617**</td>
<td>19.6 ± 0.94*</td>
<td>13.0 ± 1.3**</td>
<td>39.0 ± 1.46**</td>
</tr>
<tr>
<td>IV</td>
<td>200 mg/kg LPME + KBrO₃</td>
<td>30.83 ± 1.08**</td>
<td>1.6 ± 0.511</td>
<td>18.5 ± 0.204</td>
<td>7.66 ± 1.76</td>
<td>26.0 ± 1.06**</td>
</tr>
</tbody>
</table>

Mean ± SE (n=6). * and ** indicate significance from the control group at P<0.05 and P<0.01 probability level. ++ indicate significance from the KBrO₃ group at P<0.05 and P<0.01 probability level.

Table 3. Effect of methanolic fraction of *L. procumbens* on serum urea, direct bilirubin, total bilirubin and urobilinogen in rat.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Direct bilirubin (mg/dl)</th>
<th>Total bilirubin (mg/dl)</th>
<th>Urea (mg/dl)</th>
<th>Urobilinogen (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>3.85 ± 0.075**</td>
<td>1.99 ± 0.012**</td>
<td>1.46 ± 0.22</td>
<td>51.33 ± 1.05**</td>
</tr>
<tr>
<td>II</td>
<td>20 mg/kg KBrO₃</td>
<td>7.72 ± 0.09**</td>
<td>3.67 ± 0.021**</td>
<td>17.67 ± 1.89</td>
<td>82.00 ± 2.24**</td>
</tr>
<tr>
<td>III</td>
<td>100 mg/kg LPME + KBrO₃</td>
<td>5.54 ± 0.07*</td>
<td>2.43 ± 0.0194*</td>
<td>7.3 ± 0.929**</td>
<td>63.33 ± 1.94**</td>
</tr>
<tr>
<td>IV</td>
<td>200 mg/kg LPME + KBrO₃</td>
<td>4.01 ± 0.09**</td>
<td>2.03 ± 0.029</td>
<td>1.667 ± 0.508</td>
<td>52.50 ± 1.38**</td>
</tr>
</tbody>
</table>

Mean ±SE (n=6). * and ** indicate significance from the control group at P<0.05 and P<0.01 probability level. ++ indicate significance from the KBrO₃ group at P<0.05 probability level.

Table 4. Effect of methanolic fraction of *L. procumbens* on serum nitrite, creatinine and creatinine clearance in rat.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Creatinine (mg/dl)</th>
<th>Creatinine clearance (ml/min)</th>
<th>Nitrite (µM/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>20.50 ± 1.34**</td>
<td>1.2±0.02**</td>
<td>31.83 ± 1.56**</td>
</tr>
<tr>
<td>II</td>
<td>20 mg/kg KBrO₃</td>
<td>43.50 ± 1.89**</td>
<td>0.79 ± 0.034**</td>
<td>70.67 ± 1.84**</td>
</tr>
<tr>
<td>III</td>
<td>100 mg/kg LPME + KBrO₃</td>
<td>30.00 ± 1.77****</td>
<td>0.98 ± 0.029**</td>
<td>40.33 ± 2.06****</td>
</tr>
<tr>
<td>IV</td>
<td>200 mg/kg LPME + KBrO₃</td>
<td>24.33 ± 1.02**</td>
<td>1.02 ± 0.014**</td>
<td>34.00 ± 1.71**</td>
</tr>
</tbody>
</table>

Mean ±SE (n=6). ** indicate significance from the control group at P<0.01 probability level. + and ++ indicate significance from the KBrO₃ group at P<0.05 and P<0.01 probability level.

increased serum profile of kidney function markers, that is, serum urea, direct bilirubin, total bilirubin and concentration of urobilinogen as compared to the control group. Serum level of urea, direct bilirubin, total bilirubin and urobilinogen were significantly (P<0.01) returned towards the control group by post-treatment of *L. procumbens* in a dose dependent manner.

Effect of *L. procumbens* on serum nitrite and creatinine in rat

Table 4 shows the changes induced by KBrO₃ in the concentration of serum nitrite, serum creatinine and creatinine clearance in rat. KBrO₃ administration notably (P<0.01) amplified the level of serum nitrite, serum creatinine and decreased significantly (P<0.01) serum creatinine clearance as compared to normal rats. Serum nitrite was significantly (P<0.01, P<0.05) reversed by administration of 200 and 100 mg/kg b.w., respectively. Serum creatinine and creatinine clearance was significantly (P<0.01) restored by administration of *L. procumbens* in rats treated with KBrO₃.

Effect of *L. procumbens* on serum total protein, serum globulin and serum albumin in rat

The results of *L. procumbens* against KBrO₃ induced changes on serum total protein, globulin and albumin are shown in Table 5. KBrO₃ treatment for 4 weeks considerably (P<0.01) reduced the serum level of total protein, globulin and albumin versus the control group. Administration of various fractions of *L. procumbens* erased the toxication of KBrO₃ thereby, increasing the level of serum total protein, globulin and albumin in a dose dependent way.

**DISCUSSION**

KBrO₃ cause renal cell and thyroid carcinomas in rats, hamsters and mice when exposed chronically (Kurokawa et al., 1983). It has been investigated that KBrO₃ produces...
Table 5. Effect of methanolic fraction of *L. procumbens* on serum total protein, serum globulin and serum albumin in rat.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Albumin (mg/dl)</th>
<th>Globulin (mg/dl)</th>
<th>Total protein (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Control</td>
<td>34.07 ± 1.73**</td>
<td>23.10 ± 2.16**</td>
<td>57.17 ± 1.26**</td>
</tr>
<tr>
<td>II</td>
<td>20 mg/kg KBrO₃</td>
<td>16.67 ± 1.74**</td>
<td>19.07 ± 1.86**</td>
<td>35.74 ± 1.18**</td>
</tr>
<tr>
<td>III</td>
<td>100 mg/kg LPME+ KBrO₃</td>
<td>23.03 ± 1.21****</td>
<td>20.06 ± 2.03****</td>
<td>43.09 ± 1.38****</td>
</tr>
<tr>
<td>IV</td>
<td>200 mg/kg LPME+ KBrO₃</td>
<td>34.16 ± 1.86**</td>
<td>23.50 ± 2.11****</td>
<td>57.66 ± 1.76**</td>
</tr>
</tbody>
</table>

Mean ± SEM (n=6). * and ** indicate significance from the control group at P<0.05 and P<0.01 probability level. ++ indicate significance from the KBrO₃ group at P<0.01 probability level.

free oxygen radicals which causes oxidative stress and DNA damages (Umemura et al., 1998). KBrO₃ causes nephrotoxicity and hepatotoxicity; decreases the tissue soluble proteins, antioxidant enzymes. The decrease of antioxidant enzymes are due to reactive oxygen species (ROS) produced by metabolism of KBrO₃. KBrO₃ depleted glutathione (GSH) content in various tissues which causes decrease in phase II metabolizing enzymes like glutathione peroxidase (GSH-Px) and glutathione reductase (GSR). It also increases thiobarbituric acid reactive substances (TBARS) contents, causes lipid peroxidation and disrupts liver profile including gamma-glutamyltransferase (γ-GT), alkaline phosphatase (ALP) and protein concentration (Farombi et al., 2002). Results of the present study revealed that *L. procumbens* showed significant protection against KBrO₃-induced renal toxicity in rat. Physical analysis of urine analysis may provide information regarding the status of kidney function and acid base balance (Khan et al., 2009; Khan et al., 2010a, b). Similar effects are reported by other scientist (Ogeturk et al., 2005; Simerville et al., 2005; Khan et al., 2010b). Specific gravity and pH of urine correlates with urine osmolality and was affected (Khan et al., 2009). Data of the present investigation revealed that KBrO₃ significantly increased urine protein, RBC and WBC showing renal injuries that half kidney is damage (Bhattacharya et al., 2005). The glomerular capillary wall is permeable only to substances with a low molecular weight. Once filtered, low-molecular-weight proteins are reabsorbed and metabolized by the proximal tubule cells. High level of proteinuria and haematuria in urine of this study showed the nephrotoxicity induced with KBrO₃ (Ogawa et al., 1992). The present study revealed that oral administration of *L. procumbens* significantly improved creatinine and uricinogen, and decreased the elevated levels of proteinuria and haematuria. Present study revealed that administration of KBrO₃ caused marked impairment in renal function along with significant oxidative stress in the kidneys. Serum creatinine, uricinogen, BUN, total bilirubin and direct bilirubin concentrations were significantly higher in the KBrO₃ treated rats which are consistent with lower creatinine clearance (Adewole et al., 2007; Bhadauria et al., 2008). *L. procumbens* significantly improved creatinine clearance and decreased the elevated levels of creatinine, BUN, total bilirubin and bilirubin. In addition, elevated level of urinary albumin and reduced level of serum albumin in KBrO₃ treated rats might have resulted from remarkable leakage due to injuries in glomeruli and tubules (Adewole et al., 2007). Results of this study show protective effect on kidney urination function which might be due to the presence of some bioactive compound in plant extract. Further investigation is in process to identify this bioactive compound from *L. procumbens* extract.

REFERENCES


