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Full Length Research Paper

Alteration of renal function by potassium bromate ($KBrO_3$): Protective effects of *Launaea procumbens*

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Potassium bromate ($KBrO_3$) is a nephrotoxin which causes renal oxidative stress and cancer in experimental animal. In the present study, nephroprotective effects of *Launaea procumbens* against $KBrO_3$ 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 $KBrO_3$ (20 mg/kg b.w., i.p.), groups III and IV were administered with $KBrO_3$ 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 $KBrO_3$ 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 ($KBrO_3$), *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, vaccent 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 gonorrhoea (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, vanllic acid, synergic 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. procumben* on urine function of kidney damaged

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Table 1. Effect of methanolic fraction of *L. procumbens* on urine profile in rat.

Group	Treatment	Specific gravity	pH	Creatinine (mg/dl)	Creatinine clearance (ml/min)	Urea (mg/dl)
I	Control	1.02 ± 0.00^{++}	7.0±0.00^{++}	40.50 ± 1.34^{++}	2.04 ± 0.012^{++}	103.5 ± 1.73^{++}
II	20 mg/kg $KBrO_3$	1.31 ± 0.0427**	6.03±0.080**	63.50 ± 1.89**	0.91 ± 0.08**	208.50 ± 2.78**
III	100 mg/kg LPME + $KBrO_3$	1.29 ± 0.047**	6.55±0.061^{**++}	3.00 ± 1.77^{**++}	1.3 ± 0.059^{**++}	120.17 ± 2.52^{*++}
IV	200 mg/kg LPME + $KBrO_3$	1.17 ± 0.021^{**++}	6.81±0.079^{++}	44.33 ± 1.02^{++}	1.99 ± 0.035^{++}	105.17 ± 1.45^{++}

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 $KBrO_3$ group at $P<0.01$ probability level.

induced by potassium bromate ($KBrO_3$).

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 $KBrO_3$ 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, Orgenics, France) and standard AMP diagnostic kits (Stattogger 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 (Stattogger 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 $KBrO_3$ 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 $KBrO_3$ 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 $KBrO_3$ induced toxicity on urinary protein, albumin, urobilinogen, RBCs and WBCs level in rat are shown in Table 2. Treatment of $KBrO_3$ 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 $KBrO_3$ 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. $KBrO_3$ 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.

Group	Treatment	Albumin (mg/dl)	RBC/µl	WBC/µl	Urobilinogen (mg/dl)	Total protein (mg/dl)
I	Control	27.50 ± 1.41^{++}	0.00 ± 0.00^{++}	17.O7 ± 1.2^{++}	5.17 ± 1.26 $^{++}$	24.217 ± 0.94^{++}
II	20 mg/kg $KBrO_3$	67.33 ± 4.46**	19.33 ± 1.54**	25.0 ± 1.39**	35.74 ± 1.18**	61.0 ± 1.18**
III	100 mg/kg LPME + $KBrO_3$	39.67 ± 2.50**$^{++}$	6.7 ± 0.617**$^{++}$	19.6 ± 0.94*$^{++}$	13.0 ± 1.3**$^{++}$	39.0 ± 1.46**$^{++}$
IV	200 mg/kg LPME + $KBrO_3$	30.83 ± 1.08**$^{++}$	1.6 ± 0.511^{++}	18.5 ± 0.204^{++}	7.66 ± 1.76^{++}	26.0 ± 1.06^{++}

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 $KBrO_3$ 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.

Group	Treatment	Direct bilirubin (mg/dl)	Total bilirubin (mg/dl)	Urea (mg/dl)	Urobilinogen (mg/dl)
I	Control	3.85 ± 0.075^{++}	1.99 ± 0.012^{++}	1.46 ± 0.22^{++}	51.33 ± 1.05^{++}
II	20 mg/kg $KBrO_3$	7.72 ± 0.09**	3.67 ± 0.021**	17.67 ± 1.89**	82.00 ± 2.24**
III	100 mg/kg LPME + $KBrO_3$	5.54 ± 0.07*$^{++}$	2.43 ± 0.0194*$^{++}$	7.3 ± 0.929**$^{++}$	63.33 ± 1.94**$^{++}$
IV	200 mg/kg LPME + $KBrO_3$	4.01 ± 0.09^{++}	2.03 ± 0.029^{++}	1.667 ± 0.508^{++}	52.50 ± 1.38^{++}

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<0.01 probability level.

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

Group	Treatment	Creatinine (mg/dl)	Creatinine clearance (ml/min)	Nitrite (µM/ml)
I	Control	20.50 ± 1.34^{++}	1.2±0.02^{++}	31.83 ± 1.56^{++}
II	20 mg/kg $KBrO_3$	43.50 ± 1.89**	0.79 ± 0.034**	70.67 ± 1.84**
III	100 mg/kg LPME + $KBrO_3$	30.00 ± 1.77**$^{++}$	0.98 ± 0.029^{++}	40.33 ± 2.06**$^{++}$
IV	200 mg/kg LPME + $KBrO_3$	24.33 ± 1.02^{++}	1.02 ± 0.014^{++}	34.00 ± 1.71^{++}

Mean ±SE (n=6). ** indicate significance from the control group at *P*<0.01 probability level. + and ++ indicate significance from the $KBrO_3$ 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 $KBrO_3$ in the concentration of serum nitrite, serum creatinine and creatinine clearance in rat. $KBrO_3$ 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 $KBrO_3$.

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

The results of *L. procumbens* against $KBrO_3$ induced changes on serum total protein, globulin and albumin are shown in Table 5. $KBrO_3$ 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 $KBrO_3$ thereby, increasing the level of serum total protein, globulin and albumin in a dose dependent way.

DISCUSSION

$KBrO_3$ cause renal cell and thyroid carcinomas in rats, hamsters and mice when exposed chronically (Kurokawa et al., 1983). It has been investigated that $KBrO_3$ produces

Table 5. Effect of methanolic fraction of L. procumbens on serum total protein, serum globulin and serum albumin in rat.

Group	Treatment	Albumin (mg/dl)	Globulin (mg/dl)	Total protein (mg/dl)
I	Control	34.07 ± 1.73^{++}	23.10 ± 2.16^{++}	57.17 ± 1.26 $^{++}$
II	20 mg/kg $KBrO_3$	16.67 ± 1.74**	19.07 ± 1.86**	35.74 ± 1.18**
III	100 mg/kg LPME+ $KBrO_3$	23.03 ± 1.21**$^{++}$	20.06 ± 2.03*$^{++}$	43.09 ± 1.38**$^{++}$
IV	200 mg/kg LPME+ $KBrO_3$	34.16 ± 1.86^{++}	23.50 ± 2.11*$^{++}$	57.66 ± 1.76^{++}

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). $KBrO_3$ 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 $KBrO_3$. $KBrO_3$ 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 $KBrO_3$-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 $KBrO_3$ 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 proteinurea and haematuria in urine of this study showed the nephrotoxicity induced with $KBrO_3$ (Ogawa et al., 1992).The present study revealed that oral administration of *L. procumbens* significantly improved creatinine and urobilinogen, and decreased the elevated levels of proteinuria and haematuria. Present study revealed that administration of $KBrO_3$ caused marked impairment in renal function along with significant oxidative stress in the kidneys. Serum creatinine, urobilinogen, BUN, total bilirubin and direct bilirubin concentrations were significantly higher in the $KBrO_3$ 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 $KBrO_3$ 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

Adewole SO, Salako AA, Doherty OW, Naicker T (2007). Effect of melatonin on carbon tetrachloride-induced kidney injury in Wistar rats. Afr. J. Biomed. Res., 10: 153-164.

Ahmad M, Khan MA, Manzoor S, Zafar M, Sultana S (2006). Check list of medicinal flora of Tehsil Isakhel, District Mianwali Pakistan. Ethnobot. Leaflets, 10: 41-48.

Bhadauria M, Nirala, KS, Shukla S (2008). Multiple treatment of *Propolis* ameliorates carbon tetrachloide induced liver injuries in rats. Food Chem. Toxicol., 46: 2703-2712.

Bhattacharya H, Lun L, Gomez R (2005). Biochemical effects to toxicity of CCl_4 on rosy barbs (*Puntius conchonius*). Our Nature, 3: 20-25.

Farombi EO, Alabi MC, Akuru TO (2002). Kolaviron modulates cellular redox status and impairment of membrane protein activities induced by potassium bromate ($KBrO_3$) in rats. Pharm. Res., 45: 63-68.

Khan MR, Rizvi W, Khan GN, Khan RA, Sheen S (2009). Carbon tetrachloride-induced nephrotoxicity in rats: Protective role of *Digera muricata.* J. Ethnopharmacol., 122: 91-99.

Khan RA, Khan MR, Sahreen S (2010a). Evaluation of *Launea procumbens* use in renal disorders: a rat model. J. Ethnopharmacol., 128: 452-461.

Khan RA, Khan MR, Sahreen S, Bukhari J (2010b). Prevention of CCl_4-induced nephrotoxicity with *Sonchus asper* in rat. Food Chem. Toxicol., 23: 1304-1321.

Kurokawa Y, Hayashi Y, Maekawa A, Takahashi M, Kokubo T, Odashima S (1983). Carcinogenicity of potassium bromate administrated orally to F344 rats. J. Natl. Cancer Inst., 71: 965-972.

Ogawa M, Mori T, Mori Y, Ueda S, Azemoto R, Makino Y, Wakashin Y, Ohto M, Wakashin M, Yoshida H (1992). Study on chronic renal injuries induced by carbon tetrachloride: selective inhibition of the nephrotoxicity by irradiation. Nephron, 60: 68-73.

Ogeturk M, Kus I, Colakoglu N, Zararsiz I, Ilhan N, Sarsilmaz M (2005). Caffeic acid phenyl ester protects kidney against carbon tetrachloride toxicity in rats. J. Ethnopharmacol., 97: 273-280.

Parikh J, Chanda S (2006). Screening of aqueous and alcoholicextracts of some Indian medicinal plants for antibacterial activity. Indian J. Pharm. Sci., 68: 835-838.

Simerville JA, Maxted WC, Pahira JJ (2005). Urinalysis: A Comprehensive Review. Am. Fam. Physician, 71: 1153-1162.

Tyler V (1994). Herbs of choice. The therapeutic use of phytomedicinals. New York: Haworth Press.3rd Edition.

Umemura T, Takagi A, Sai K, Hasegawa R, Kurokawa Y (1998). Oxidative DNA damage and cell proliferation in kidneys of male and female rats during 13-weeks exposure to potassium bromat e (KBrO3). Arch. Toxicol., 72: 264-269.

Yousaf Z, Shainwari ZK, Ali SM (2004). Medicinally important flora of Dhibbia Karsal Village (Mianwali District Punjab). Asian J. Plant Sci., 3: 757-762.