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Full Length Research Paper

Amelioration of kidney function markers by *Sonchus asper* butanolic extract against $KBrO_3$-induced toxicity in rat

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The protective effects of *Sonchus asper* butanol extract against $KBrO_3^-$ induced oxidative damage in kidney of rat were determined in this study. 24 Sprague-Dawley male rats (180 to 200 g) were divided into 4 groups. Group I was given saline (1 ml/kg b.w., 0.85% NaCl) and DMSO (1 ml/kg b.w.); group II was treated with $KBrO_3$ (20 mg/kg b.w., i.p.); group III and IV administered with $KBrO_3$ and after 48 h with *Sonchus asper* butanolic extract (SBE) (100; 200 mg/kg b.w.). All the treatments were given twice a week for five weeks. The results revealed that $KBrO_3$ induced oxidative stress as evidenced by the significant alteration in physical and serum marker of kidney which were restored with SBE dose dependently revealed that *S. asper* butanol extract can protect the kidney against $KBrO_3$ mediated oxidative damage.

Key words: *Sonchus asper*, $KBrO_3$, oxidative stress, physical analysis, serum markers.

INTRODUCTION

Potassium bromate ($KBrO_3$) molecular weight 166 g/mol is an oxidizing agent. $KBrO_3$ has been used in industries for the formation of hair solution and cosmetics. Potassium bromate is formed as by product during ozanization of water, causes infections and has been classified as 2B group toxic chemical a probable human carcinogen (IARC, 1986). $KBrO_3$ causes renal cell and thyroid carcinomas in rats, hamsters and mice when exposed chronically (Kurokawa et al., 1983). It has been investigated that potassium bromate produces 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, alteration in physical parameters of urine and disturb serum markers (Khan et al., 2011). The alteration of kidney function markers are due to reactive oxygen species (ROS) produced by metabolism of potassium bromate. Potassium bromate increased urinary protein, urobilinogen, RBCs, WBCs and albumin concentration. It also increases thiobarbituric acid-reactive substances (TBARS) contents, causes lipid peroxidation and disrupts liver profile including γ-GT, ALP and protein concentration (Farombi et al., 2002). Medicinal plants play important role in recovery of various diseases (Khan et al., 2009, 2010a, b, 2011a, b; Sahreen et al., 2010, 2011). *S. asper* also constitutes important food components including protein, sugar and life saving elemental contents like calcium, potassium, phosphorus and zinc (Afolayan and Jimoh, 2008; Khan et al., 2010c). Chemical characterization of *S. asper* shows that it contains polyphenolic compounds and sesquiterpene lactones glycosides (Helal et al., 2000). Nutritional analysis of *S. asper* showed the presence of vitamin C and phenolic constituents (Guil-Guerrero et al., 1998). Flavonoids, ascorbic acid and carotenoids possess antioxidant, anticancer, anti-inflammatory properties while, sesquiterpene lactones glycosides have anti

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Table 1. Effect SBE on urine pH, specific gravity, creatinine, creatinine clearance and urea in rat.

Treatment	pH	Specific gravity	Urea (mg/dl)	Creatinine (mg/dl)	Creatinine clearance (ml/min)
Control	7.20±0.00++	1.03±0.02++	83.0±2.03++	40.50±1.34++	2.4±1.3++
20 mg/kg $KBrO_3$	6.03±0.08**	1.41±0.04 **	185.30±3.9**	73.50±1.89**	1.21±1.89**
100 mg/kg SBE *r*+ $KBrO_3$	6.5±0.06++	1.2±0.07++	141.1±3.52++	53.00±1.77++	2.3±1.7++
200 mg/kg SBE + $KBrO_3$	6.9±0.07++	1.03±0.021++	98.10±3.02++	41.33±1.02++	2.5±1.02++

Mean ± SE (n=6 number). ** indicate significance from the control group $p<0.01$ probability level. ++ indicate significance from the $KBrO_3$ group at $p<0.01$ probability level.

oxidant antiinflammatory activities and also play important role in cardiovascular disfunction. In the present study, the effect of butanol extract is evaluated against $KBrO_3^-$ induced alteration of kidney function test.

MATERIALS AND METHODS

Plant collection and extraction

S. asper collection, extraction was carried out by the protocol as used by Khan et al. (2010b) and fractionated with butanol to get butanol extract (SBE), dried and stored at refrigerator for further investigation.

Experimental design

24 male Sprague Dawley rats (180 to 200 g) were procured from National Institute of Health Islamabad and were kept in ordinary cages at room temperature of 25±3°C with a 12 h dark/light cycle and accessed to standard feed and water according to the study protocol approved by Ethical committee of Quaid-i-Azam University Islamabad. Rats were equally divided into 4 groups (6 rats each). Group 1 was remained untreated while Group II was 20 mg/kg $KBrO_3$ in saline. Group III and IV were given intragastrically (100; 200 mg/kg body weight) SBE after 48 h of $KBrO_3$ treatment. All these treatments were given for five weeks. After the completion of experiment urine and blood was collected from animals and stored at -70°C for various enzymatic and other biochemical studies.

Assessment of physical parameters

Physical analysis of urine

Before sacrifice rats were kept individually in metabolic cages for 24 h to collect their urine for estimation of renal function tests. Urine samples were assayed for pH, Sp.Gr, urea, urinary creatinine, urinary creatinine clearance, urinary protein, albumin, urobilinogen, RBCs and WBCs count was determined 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 conformed by using pH meter.

Serum analysis

Analysis of serum includes blood urea (BUN), serum nitrite, serum creatinine, serum creatinine clearance, serum total protein, globulin, and albumin was estimated by using standard AMP diagnostic kits (Stattogger Strasse 31b 8045 Graz, Austria).

Statistical analysis

To determine the treatment effects one way analysis of variance was carried by computer software SPSS 13.0. Level of significance among the various treatments was determined by LSD at 0.05% level of probability.

RESULTS

Effect of SBE on urine pH, specific gravity, creatinine, creatinine clearance and urea in rat

Reactive oxygen species (ROS) especially nephrotoxic chemicals effects the urinary profile of kidney. Table 1 shows the changes in renal profile including urinary pH, specific gravity, creatinine, creatinine clearance and urea level. Administration of nephrotoxic $KBrO_3$ treatment significantly ($P<0.01$) increased the level of specific gravity, urea, creatinine, while reduced ($P<0.01$) the urinary pH and creatinine clearance than control. SBE attenuated the $KBrO_3$ intoxication, and reduced ($P<0.01$) the specific gravity, urea, creatinine while increased the pH and creatinine clearance of urine.

Effect of SBE on urinary protein, albumin, urobilinogen, RBCs, WBCs count in rat

Effect of SBE 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 compare to non treated control group. Post-administration of SBE significantly augmented ($P<0.01$) the toxicity of $KBrO_3$ and the urinary level of these parameters returned towards the control group.

Effect of SBE on serum urea, direct bilirubin, total bilirubin and urobilinogen in rat

Effects of SBE versus $KBrO_3^-$ treated rat, changes in serum urea, direct, total bilirubin and urobilinogen as well

Table 2. Effect SBE on urea, RBC, WBC, protein, albumin, urobilinogen in rat.

Treatment	RBC/µl	WBC/µl	Protein (mg/dl)	Albumin (mg/dl)	Urobilinogen (mg/dl)
Control	0.00±0.00++	12.00±1.41++	42.83±2.26 ++	18.17±2.76++	2.47±0.22++
20 mg/kg $KBrO_3$	19.33±1.54**	60.33±4.46**	98.00±1.18**	34.67±1.86**	15.67±1.89**
100 mg/kg SBE *r*+ $KBrO_3$	6.783±0.67**++	29.67±2.5**++	68.67±1.3**++	26.20±2.7++	7.30±0.92++
200 mg/kg SBE + $KBrO_3$	1.683±0.51++	13.83±1.08++	44.67±1.76++	20.51±2.11++	2..67±0.15++

Mean ± SE (n=6 number). ** indicate significance from the control group at *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 SBE on serum urea, direct bilirubin, total bilirubin and urobilinogen in rat.

Treatment	Serum urea (mg/dl)	Direct bilirubin (mg/dl)	Total bilirubin (mg/dl)	Urobilinogen (mg/dl)
Control	60.10±2.05++	3.96±0.07++	1.99±0.02++	2.47±0.22++
20 mg/kg $KBrO_3$	99.01±3.14**	4.92±0.09**	3.67±0.02**	17.67±1.89**
100 mg/kg SBE *r*+ $KBrO_3$	76.33±1.94**++	4.42±0.07++	2.45±0.04++	7.00±0.92**++
200 mg/kg SBE + $KBrO_3$	65.50±1.38 ++	4.01±0.09++	2.0±0.02++	2..67±0.15++

Mean ±SE (n=6 number). ** indicate significance from the control group *p<0.01* probability level. ++ indicate significance from the $KBrO_3$ group at *p*<0.01 probability level.

Table 4. Effect of SBE on serum nitrite, creatinine and creatinine clearance in rat.

Treatment	Nitrite (µM/ml)	Creatinine (mg/dl)	Creatinine clearance(ml/min)
Control	51.0±1.14++	36.50±0.84++	1.93±0.013 ++
20 mg/kg $KBrO_3$	82.12±1.44**	80.50±2.00**	0.99±0.08**
100 mg/kg SBE *r*+ $KBrO_3$	68.509±1.61**+	53.00±1.77**++	1.47±0.07**++
200 mg/kg SBE + $KBrO_3$	55.26±1.86++	40.33±2.42++	1.70±0.05++

Mean ±SE (n=6 number). ** indicate significance from the control group at *p*<0.01 probability level. ++ indicate significance from the $KBrO_3$ group at *p*<0.01 probability level.

as in non treated control, DMSO treated rats are shown in Table 3. $KBrO_3$ treatment to rats significantly (P<0.01) increased serum profile of kidney viz; serum urea, direct, total bilirubin and concentration of urobilinogen in serum as compared to the control group.

Serum level of urea, direct, total bilirubin and urobilinogen were significantly (P<0.01) returned towards the control group by post-treatment of SBE depend on quantity of dose.

Effect SBE on serum nitrite, creatinine and creatinine clearance 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 comparatively to normal rats. Serum nitrite was significantly (P<0.01) reversed by administration of 200 mg/kg b.w and by (P<0.05) significance at 100 mg/kg b.w. Serum creatinine and creatinine clearance was significantly (P<0.01) restored administration of SBE in rats treated with $KBrO_3$.

Effect of SBE on serum total protein, serum globulin and serum albumin in rat

The results *S. asper* against $KBrO_3$ administration on the changes of serum total protein, globulin and albumin are shown in Table 5. $KBrO_3$ treatment for 5 weeks considerably (P<0.01) reduced the serum level of total protein, globulin and albumin versus the control group. Administration of various concentrations of SBE erased the toxication of $KBrO_3$ thereby increased the level of serum total protein, globulin and albumin in a dose

Table 5. Effect of SBE on serum total protein, serum globulin and serum albumin in rat.

Treatment	Total serum protein (mg/dl)	Serum globulin (mg/dl)	Serum albumin (mg/dl)
Control	42.83±2.26 ++	24.67±1.33++	18.17±2.76++
20 mg/kg $KBrO_3$	23.00±1.18**	12.33±1.54**	10.67±1.86**
100 mg/kg SAME+ $KBrO_3$	34.67±1.38**++	18.67±1.28**++	16.00±2.73++
200 mg/kg SAME+ $KBrO_3$	39.67±1.76++	22.17±1.42++	17.50±2.11++

Mean ± SEM (n=6 number). ** indicate significance from the control group at $p<0.01$ probability level. ++ indicate significance from the $KBrO_3$ group at $p<0.01$ probability level.

dependent way.

DISCUSSION

Results of the present project revealed that significant protection of SBE 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). During normal condition urobilinogen is not excreted into the urine unless any pathogenesis. Urobilinogen; is the end product of conjugated bilirubin after it has passed through the bile ducts and been metabolized in the intestine. The presence of high levels of urobilinogen, urea, creatinine and albumin in urine is the main indication of kidney injuries induced through $KBrO_3$ treatment (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). Our results showed that $KBrO_3$ significantly increased urine protein, RBC and WBC showing renal injuries. Glomerular haematuria is typically associated with erythrocyte cases, dysmorphic red blood cells and significant proteinuria. The serum creatinine level does not rise until at least half of the kidney nephrons are damaged or destroyed (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 different concentrations 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, direct bilirubin concentrations were significantly higher in the chemicals treated rats which are consistent with lower creatinine clearance (Adewole et al., 2007; Bhadauria et al., 2008). SBE significantly improved creatinine clearance and decreased the elevated levels of creatinine, BUN, total bilirubin and direct 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. Other studies of Adewole et al. (2007) and Khan et al. (2009) were reported during their plant extracts.

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