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Full Length Research Paper

Protective effect of *Launaea procumbens* against $KBrO_3$ induced nephrotoxicity in rats

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Launaea procumbens **is traditionally used in the treatment of renal dysfunction. In the present study, protective effects of *L. procumbens* against $KBrO_3$-induced nephrotoxicity in rat were determined. In this study, 24 male albino rats (195 to 200 g) were equally 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 $KBrO_3$ (30 mg/kg b.w., i.p.); Groups III and IV administered with $KBrO_3$ and after 48 h with *L. procumbens* (100; 200 mg/kg b.w.). All the treatments were given twice a week for 6 weeks. The results revealed that $KBrO_3$ induced oxidative stress as evidenced by the significant depletion ($P < 0.01$) of antioxidant enzymes (catalase CAT, superoxide dismutase, SOD, glutathione-S-transferase, GST, glutathione reductase; GSR, glutathione peroxidase GSH-px and quinone reductas, QR) and glutathione contents while enhanced markedly ($P < 0.01$) tissue nitrite, lipid peroxidation and hydrogen peroxide level in kidney. Co-administration revealed that *L. procumbens* methanol extract significantly ($P < 0.01$) protect the liver against $KBrO_3$ mediated oxidative damage by restoring activity of antioxidant enzyme and lipid peroxidation which might be due to the presence plant bioactive constituents.**

Key words: *Launaea procumbens,* $KBrO_3$, oxidative stress, superoxide dismutase (SOD), glutathione peroxidase (GSH).

INTRODUCTION

The imbalance in prooxidant and antioxidant leads oxidative stress causes various disorders and degenerative processes such as aging, immuno- deficiencies, neurological disorder, and cancer. The major cellular sources responsible for the generation of damage-inducing reactive oxygen species (ROS), such as $\cdot O_2^-$, H_2O_2 and $\cdot OH$, are mitochondria and peroxisomes (Kang et al., 2003). Free radical initiate lipid peroxidation of cell membranes and oxidative damage of proteins, which in turn cause changes in membrane fluidity, disruption of microsomes, lysosomes, accumulation of peptide fragments and cross linked protein aggregates (Pietrangelo, 2003). This ultimately leads to cellular dysfunction and eventually to apoptosis or necrosis. $KBrO_3$ is a well known nephrotoxin which cause depletion of antioxidant as well as disturb serum marker enzymes and cholesterol profile (Khan et al., 2011).

Medicinal plants have been used for centuries as remedies for human diseases. They have immensely contributed to the health needs of humans throughout their existence. Even today, almost one quarter of prescribed medicines in the world control ingredients from plant origin (Iqbal and Rahman, 2004). Although modern drugs are effective in preventing kidney disorders, their use is often limited because of their side effects. Nowadays, it is being realized that herbs can protect the kidney by providing an integrated structure of nutritional substances mainly phytochemicals which help in restoring and maintaining balanced body systems (Hertog et al., 1993).

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Launaea procumbens (Asteraceae) found in waste places, vacant lots and in cultivated fields through out Pakistan used as cattle's foods and as washing agent (Wazir et al., 2007) possesses salicylic acid, vanillic acid, synergic acid, 2-methyl-resercinol and gallic acid. This plant possesses antifungal, nematodcidal and allelopathic properties (Shaukat et al., 2003). Ethnopharmacologically, *L. procumbens* is used in the treatment of kidneys, liver and in sexual disorders. Methanolic extract of *L. procumbens* is found to be composed of flavonoids, terpenoids, tannins, cardiac glycosides and phlobatannins (Khan et al., 2010b). Antioxidant activity of *L. procumbens* against carbon tetrachloride (CCl_4)-induced nephrotoxicity has been well documented, and the activity has been ascribed to flavonoid constituents (Khan et al., 2010b). In this study, we have investigated the nephroprotective effects of the methanolic extract of *L. procumbens* against the injuries induced with $KBrO_3$, a well known nephrotoxin.

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 two weeks, chopped, and grinded mechanically. 2 kg powder of *L. procumbens* was extracted in 80% methanol (4 L) 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 4°C for further *in vivo* investigations.

Animals and experimental design

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±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 remained untreated while Group II received 20 mg/kg bw $KBrO_3$ in saline on Monday and Thursday. Groups III and IV were given intragastrically (100 and 200 mg/kg body weight, respectively) LME after 48 h of $KBrO_3$ treatment (Wednesday and Saturday). All these treatments were given for four weeks. After the completion of the experiment, animals were sacrificed and kidneys were washed with saline solution. Kidney was dried under liquid nitrogen and stored at -70°C for various enzymatic and other biochemical studies.

Nephrooxidant studies

Kidney tissue was homogenized in 10 volume of 100 mmol KH_2PO_4 buffer containing 1 mmol EDTA (pH 7.4) and centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was collected and used for the following experiments as described subsequently. Protein concentration of the supernatant of kidney tissue was determined by Lowry et al. (1951) using crystalline BSA as standard. Activity of catalase; CAT (Chance and Maehly, 1955), superoxide dismutase; SOD (Kakkar et al., 1984), glutathione-S-transferase; GST (Habig et al., 1974), glutathione reductase; GSR (Carlberg and Mannervik, 1975), glutathione peroxidase GSH-px (Mohandas et al., 1984) and quinone reductase; QR was determined according to Benson et al. (1980).

Reduced glutathione (GSH) assay

Reduced glutathione was estimated by the method of Jollow et al. (1974) by using 1,2-dithio-bis nitro benzoic acid (DTNB) as substrate. The yellow color developed was read immediately at 412 nm and expressed as µmol GSH/g tissue.

Estimation of lipid peroxidation (TBARS) assay

Kidney thiobarbituric acid-reactive substances (TBARS) were measured at 535 nm by using 2-thiobarbituric acid (2,6-dihydroxypyrimidine-2-thiol; TBA). An extinction coefficient of 156,000 M^{-1} cm^{-1} was used for calculation.

Hydrogen peroxide assay (H_2O_2)

Hydrogen peroxide (H_2O_2) was assayed by H_2O_2-mediated horseradish peroxidase dependent oxidation of phenol red. 2.0 ml of homogenate sample was suspended in 1.0 ml of solution containing phenol red (0.28 nmol), horse radish peroxidase (8.5 units), dextrose (5.5 nmol) and phosphate buffer (0.05 mol; pH 7.0) and were incubated at 37°C for 60 min. The reaction was stopped by the addition of 0.01 ml of NaOH (10 N) and then centrifuged at 800 × g for 5 min. The absorbance of the supernatant was recorded at 610 nm against a reagent blank. The quantity of H_2O_2 produced was expressed as nmol H_2O_2/min/mg tissue based on the standard curve of H_2O_2 oxidized phenol red.

Nitrite assay

To determine the nitrite contents in renal tissues supernatant was collected after deproteinized with NaOH and $ZnSO_4$ with centrifugation at 6400 × g for 20 min. Griess reagent was used to blank the spectrophotometer at 540 nm and supernatant was added. Nitrite concentration was calculated using a standard curve for sodium nitrite.

Statistical analysis

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

RESULTS

Effect of LME on kidney protein, CAT, POD and SOD activity in rat

The results regarding the protective effects of *L. procumbens* against the toxic affect of $KBrO_3$ in rat on kidney protein and activities of antioxidant enzymes such as CAT, POD and SOD are shown in Table 1.

Table 1. Effect of LME on kidney protein, CAT, POD and SOD activity in rat

Group	Protein (µg/mg tissue)	CAT (U/min)	POD (U/min)	SOD (U/mg protein)
I	1.23±0.103++	2.0442±0.0819++	12.05±1.54++	4.667±0.514++
II	2.701±0.057**	1.3442±0.0819**	4.075±0.859**	2.008±0.286**
III	1.98±0.0386**++	1.4325±0.0471++	8.375±0.579++	4.242±0.193**++
IV	1.55±0.0394++	1.8842±0.0579++	9.375±0.591++	4.775±0.197++

Results are expressed as Mean±SE (n=6 number); *, **, Significance from the control group at $P<0.05$ and $P<0.01$ probability level, respectively; $^{++}$, significance from the $KBrO_3$ group at $P<0.05$ and $P<0.01$ probability level.

Table 2. Effect of LME on kidney GSH-Px, GST, GSR and QR activity in rat.

Group	GSR (nM /min/mg protein)	GSH-Px (nM /min/mg protein)	QR (nM /min/mg protein)	GST (nM /min/mg protein)
I	$123.3\pm10.3^{++}$	$45.33\pm5.65^{++}$	$82.17\pm1.08^{++}$	$170.83\pm5.11^{++}$
II	84.50±4.32**	25.35±2.08**	52.00±2.96**	133.42±8.19**
III	$98.83\pm3.86^{**++}$	$36.86\pm2.12^{**++}$	$69.50\pm1.88^{**++}$	$142.25\pm4.71^{**++}$
IV	$118.50\pm3.94^{++}$	$41.52\pm2.17^{++}$	$75.83\pm1.54^{++}$	$168.42\pm5.79^{++}$

Results are expressed Mean±SE (n=6 number); *, **. Significance from the control group at $P<0.05$ and $P<0.01$ probability level, respectively; $^{++}$, significance from the $KBrO_3$ group at $P<0.01$ probability level .

Table 3. Effect of LME on kidney TABRS, GSH, H_2O_2 and nitrite contents in rat

Group	TBARS (nM/ min/mg protein)	H_2O_2 (nM/ min/mg tissue)	GSH (µM/g tissue)	Nitrite (µM/ml)	XO (µg/min /mg protein)
I	11.86±0.25++	11.86±0.25++	0.56±0.020++	0.47±0.01++	41.98±0.08++
II	16.5±0.764**	16.5±0.764**	0.319±0.014**	0.96±0.027**	24.91±0.16**
III	13.6±1.02**++	13.6±1.02**++	0.432±0.021**++	0.67±0.012**++	36.02±0.07*++
IV	12.500±0.214++	12.500±0.214++	0.52±0.025++	0.49±0.012++	40.26±0.06++

Results are expressed Mean±SE (n=6 number); * and **, Significance from the control group at $P<0.05$ and $P<0.01$ probability level, respectively; $^{++}$, significance from the $KBrO_3$ group at $P<0.01$ probability level.

Concentration of soluble tissue protein, CAT, POD and SOD were reduced by treatment of $KBrO_3$ as comparatively to normal rats. This drop was reversed significantly ($P < 0.01$) by post-administration of LME at both 100 and 200 mg/kg b.w and the concentration of tissue soluble protein and activities of antioxidant enzymes near to control rat. The ameliorating effects might be due to the presence of flavonoids, saponins, tannins and other bioactive compounds against the toxicity of $KBrO_3$.

Effect of SME on kidney GST, GSH-Px, GSR and QR activity in rat

Effect of $KBrO_3$ and the protective effects of different concentrations of LME on tissue phase II metabolizing enzymes including; GST, GSR, GSH-Px, and QR are shown in Table 2. $KBrO_3$ treatment to rats significantly ($P < 0.01$) decreased the activities of GSH-Px, GST, GSR and QR. Administration of LME showed significant protection and recovered ($P < 0.01$) the activity of enzymes near to control rat, and increased the activities of GST, GSR and QR in a dose dependent manner.

Effect of LME on kidney TABRS, GSH, H_2O_2 and nitrite contents in rat kidney

Table 3 shows the changes of protection by LME versus the $KBrO_3$ intoxication of rat on TABRS, GSH, H_2O_2 and nitrite contents in tissue homogenate. Treatment of $KBrO_3$

considerably ($P < 0.01$) amplified the contents of TABRS, H_2O_2, nitrite concentration and significantly ($P < 0.01$) decreased the content of GSH in tissue homogenate comparatively to normal rats. Contents of TABRS was significantly decreased ($P < 0.01$) by post-administration of LME in rat treated with $KBrO_3$. Level GSH and nitrate was notably ($P < 0.01$) restored by both 100 and 200 mg/kg bw administration of LME.

DISCUSSION

Oxidative stress was characterized by increased lipid peroxidation and/or altered non-enzymatic and enzymatic antioxidant systems. Cumulative evidence suggested that various enzymatic and non-enzymatic systems have been developed by mammalian cells to cope with ROS and other free radicals (Ahmad et al., 2011; Khan et al., 2010a). Superoxide dismutase (SOD), peroxidase (POD) catalase (CAT) constitutes a mutually supportive team of defense against ROS. Oxidative insult induces reduction of antioxidant enzymes including SOD, CAT and POD which in turn caused reduction of antioxidant status (Khan et al., 2009). The endogenous antioxidant enzymes are responsible for the detoxification of deleterious oxygen radicals. CAT is a hemoprotein which catalyses the reduction of hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals (Sahreen et al., 2010). SOD constitutes an important link in the biological defense mechanism through dismutation of endogenous cytotoxic superoxide radicals to H_2O_2 (Khan et al., 2011a). $KBrO_3$ significantly reduced activities of CAT, POD and SOD, which are augmented with extract at different doses. Similar results are reported by other investigation of Khan et al. (2011b) during estimation of protective effects of Sonchus asper against $KBrO_3$ induced cardiac dysfunction. The protective effects of Carissa opaca against CCl_4 induced hepatotoxicity in rats.

GSH levels (reduced form) are important for maintaining the structural and functional integrity of different organs. The maintenance of cellular GSH levels is dependent upon the activities of glutathione reductase (GSR) and NADH. The glutathione system includes GSH, GSR, GSH-Px and GST. GSH-Px. Glutathione detoxifies toxic metabolites of drugs, regulates gene expression, apoptosis, and transmembrane transport of organic solutes (Khan et al., 2010b, 2011b). Administration of LME showed significant protection and recovered ($P < 0.01$) the activity of enzymes near to control rat; increased the activities of GST, GSR and QR in a dose dependent manner as was altered with $KBrO_3$ free radicals. Similar observations were also reported during administration of melatonin against chemically induced oxidative stress in kidneys (Adewole et al., 2007). Khan et al. (2009) documented same during estimation of Digera muricata protective effects in free radicals induced toxicity in rat kidneys.

Oxidant-induced injury to the plasma membrane of cells can evoke a wide variety of responses. Biological membranes contain a large amount of polyunsaturated fatty acids, which are particularly susceptible to peroxidative attacks to produce lipid peroxides. Lipid peroxides have been used as a sensitive indicator of oxidant-induced cell injury (Khan et al., 2011, 2011c). Oxidative stress can promote the formation of a variety of vasoactive mediators that can affect renal function directly by causing renal vasoconstriction. The increase in nitrite contents with free radicals treatment in kidney tissues might be due to direct damages which cause constriction of blood capillaries (Khan et al., 2009). These injuries further enhance the nitrite contents in kidney tissues. $KBrO_3$ considerably amplified the contents of TABRS, H_2O_2, nitrite concentration and deplete the tissue GSH. Co-treatment markedly ($P < 0.01$) reimbursed these alterations. Other results of Sahreen et al. (2011a, b) are in accordance to our study. Administration of Coriandrum sativum extracts against oxidative stress in kidneys produced similar results that support our study (Sreelatha et al., 2009).

Conclusion

Methanolic extract of *L. procumbens* showed significant nephroprotective activity against $KBrO_3$ induced alterations which might be due the presence of its bioactive constituents. Further research work on isolation and purification of bioactive constituents are in progress in our lab.

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