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Full Length Research Paper

Effect of various fractions of *Launaea procumbens* on antioxidant enzymes in rats liver: Oxidative stress induced by potassium bromate ($KBrO_3$)

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Launaea procumbens **is traditionally used in the treatment of liver dysfunction and hepatitis. In the present study, protective effects of *L. procumbens* against potassium bromate ($KBrO_3$)-induced hepatotoxicity of rat were determined. However, 36 male albino rats (180 to 200 g) were equally divided into 6 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, IV, V and VI were administered with $KBrO_3$ and after 48 h with 200 mg/kg b.w. of various fractions of *L. procumbens* twice a week for 4 weeks. Data showed that the $KBrO_3$ induced oxidative damages were caused by considerable diminution of antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione-S-transferase (GST), glutathione peroxidase (GSH-px) and glutathione reductase (GSR)] and glutathione (GSH) contents. Co-administration revealed that 200 mg/kg b.w. of various fractions of *L. procumbens* defend the liver against $KBrO_3$ mediated oxidative damage by restoring activity of antioxidant enzyme, which might be due to the presence of plant bioactive constituents.**

Key words: *Launaea procumbens*, potassium bromate ($KBrO_3$), catalase (CAT), rats.

INTRODUCTION

An imbalance in the pro-oxidant and antioxidant level can lead to oxidative stress. The oxidative stress of potassium bromate ($KBrO_3$) induces injuries in different tissues and organs through reaction with proteins, lipids and nucleic acids. Production of reactive oxygen species (ROS) due to $KBrO_3$ causes many diseases, such as cancer, ageing and diabetes mellitus (Sun, 1990). $KBrO_3$ causes oxidative damages. Its potential uses are as food additive and in cold-wave hair lotions. Other uses of $KBrO_3$ are in beverages and food products (Kurokawa et al., 1983). Renal cell tumors, carcinomas are caused due to $KBrO_3$. It also induces micronuclei formation, chromosomal anomalies *in vitro* and *in vivo* conditions (Kurokawa et al., 1990). $KBrO_3$ administration in rat cause renal tumors (Umemura et al., 1995). Delker et al. (2006) investigated the involvement of $KBrO_3$-induced oxidative stress in cancer; $KBrO_3$ was administered in rat for 2 to 100 weeks cause upregulation of certain genes involved in ion transport, cancer and oxidative stress. Similarly, $KBrO_3$ orally gavage proved the enrichment in liver as compared to kidney and testis. Soy isoflavones ameliorate the oxidative damages induced with $KBrO_3$ in rats. Concentration of glutathione (GSH), activity level of antioxidant enzymes in renal tissues was reduced with $KBrO_3$ while activity level of γ-glutamyl transpeptidase, xanthine oxidase, H_2O_2 and lipid peroxidation was enhanced. Similarly, renal profile was increased, which

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were reversed by treatment of soy isoflavones. Medicinal plants, fruits and common Pakistani vegetables play crucial role in treatment of various oxidative dysfunction (Khan et al., 2009; Khan et al., 2010a, b; Khan et al., 2011a, b; Sahreen et al., 2010; Sahreen et al., 2011). *Launaea procumbens* is a common Pakistani vegetable, used as a traditional remedy for treatment of liver. Therefore, the present study is arranged to investigate its protective role against $KBrO_3$-induced oxidative stress in male albino rats.

MATERIALS AND METHODS

Plant collection and extraction

Plant collection and extraction was carried out as previously described by Khan et al. (2010a) with some modification.

Animals and treatment

Six-week-old male Sprague-Dawley rats weighing 180±10 g were provided with food and water *ad libitum* and kept at 20 to 22°C on a 12 h light-dark cycle. We used only male rats because of their constant metabolism compared to the variation in the female physiology. All experimental procedures involving animals were conducted in accordance with the guidelines of National Institutes of Health (NIH guidelines Islamabad, Pakistan). The study protocol was approved by Ethical Committee of Quaid-i-Azam University, Islamabad. The rats were acclimatized to laboratory condition for 7 days before commencement of experiment.

Thirty-six (36) male albino rats were randomly divided into six groups (6 rats): Group I which is the normal control received only feed, Group II which is the $KBrO_3$ group received intragastric administration of $KBrO_3$ 20 mg/kg body weight (aqueous) twice a week for 4 weeks. Group III was given 200 mg/kg b.w., *L. procumbens* methanolic extract (LPME), Group IV was treated with 200 mg/kg b.w., *L. procumbens* chloroform extract (LPCE), Group V was administered with 200 mg/kg b.w., *L. procumbens* ethyl acetate extract (LPEE) and Group VI were treated with 200 mg/kg of body weight *L. procumbens* n-hexane extract (LPHE), orally twice a week for 4 weeks. At the end of 4 weeks after 24 h of the last treatment, all the animals were sacrificed; blood was drawn prior to the excision of organ. The serum was stored at -80°C after separation until it was assayed. Half of liver tissues were treated with liquid nitrogen and stored at -80°C for further enzymatic analysis.

Assessment of antioxidant profile

70 mg of tissue was homogenized in 10 vol of 100 mmol KH_2PO_4 buffer containing 1 mmol ethylene diamine tetraacetic acid (EDTA) (pH 7.4) and centrifuged at 12,000 × g for 30 min at 4°C. The supernatant was collected and used for enzymatic studies. Protein concentration of tissue supernatant was determined by the method of Lowry et al. (1951) using crystalline bovine serum albumin (BSA) as standard. Activity of catalase (CAT) (Chance and Maehly, 1955), peroxidase (POD) (Chance and Maehly, 1955), superoxide dismutase (SOD) (Kakkar et al., 1984), glutathione-S-transferase (GST) (Habig et al., 1974), glutathione peroxidase (GSH-Px) (Mohandas et al., 1984), glutathione reductase (GSR) (Carlberg and Mannervik, 1975), quinone reductase (QR) (Benson et al., 1980), GSH (Jollow et al., 1974), thiobarbituric acid reactive substances (TBARS) (Iqbal et al., 1996), H_2O_2 (Pick and Keisari, 1981) and nitrite contents in rat hepatic homogenate.

Statistical assay

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

RESULTS AND DISCUSSION

Effect of various fractions of *L. procumbens* on antioxidant enzymes of liver

Depletion of antioxidant responses has been implicated in the kidney carcinogenicity of $KBrO_3$ previously. The activities of antioxidant enzymes including SOD, CAT, GSHPx and GSH contents were significantly reduced while increased lipid peroxidation in rat following an acute exposure of $KBrO_3$. Protein, CAT, POD and SOD are an index of antioxidant system which play a key role in detoxification of ROS. Treatment of $KBrO_3$ to rats decreased ($P<0.01$) the amount of tissue soluble protein and activities of antioxidant enzymes than control. 200 mg/kg b.w various fractions of *L. procumbens* attenuated the toxicity of $KBrO_3$ and reversed the enzyme activities, near to control group as shown in Table 1. Sahreen et al. (2011) and Khan et al. (2011) reported same results with plant extracts.

Effect of various fractions of *L. procumbens* on liver GST, GSH-Px, GSR, lactate dehydrogenase (LDH) and QR activity in rat

GSH depletion which occurs due to high levels of intracellular oxygen radicals, may result in the transcriptional activation of many sulfur and methionine metabolizing genes. GSH and other sulfhydryls play a major role in the metabolism and excretion of bromate in the rat. Table 2 shows the changes in activity of GSR, GSH-px, GST and QR in various groups of the present study. 20 mg/kg b.w of $KBrO_3$ treatment to rats considerably ($P<0.01$) decreased the activity of GSR, GSH-Px, GST, lactate dehydrogenase (LDH) and QR as comparatively to normal rats. Activity of these enzymes was significantly ($P<0.01$) recovered by post-treatment of 200 mg/kg b.w various fractions of *L. procumbens* in $KBrO_3$ treated rats near to control group. The results of Khan et al. (2010c) are in accordance to our study.

Effect of various fractions of *L. procumbens* on liver GSH, TBARS, H_2O_2 and nitrite contents in rat

Generation of superoxide radicals as an effect of $KBrO_3$ intoxication would further stimulate lipid peroxidation and cellular injuries. TBARS, the final metabolite of peroxidized polyunsatured fatty acids, considered as a late biomarker of oxidative stress, not only translates

Table 1. Effect of various fractions of *L. procumbens* on liver protein, CAT, POD and SOD activity in rat.

Group	Treatment	Protein (µg/mg tissue)	CAT (U/min)	POD (U/min)	SOD (U/mg protein)
Group I	Control	2.97±0.13^{++}	19.05±1.29^{++}	17.05±1.29^{++}	36.11±2.57^{++}
Group II	20 mg/kg $KBrO_3$	1.57±0.09**	6.247±0.49**	9.247±1.220**	19.49±0.99**
Group III	200 mg/kg LPME+ $KBrO_3$	2.8±0.217^{++}	18.99±1.61^{++}	16.99±3.95^{++}	34.99±3.22^{++}
Group IV	200 mg/kg LPCE+ $KBrO_3$	2.73±0.197^{++}	17.28±1.47^{++}	17.28±3.61^{++}	29.57±2.95^{++}
Group V	200 mg/kg LPEE+ $KBrO_3$	3.1±0.160^{++}	50.19±1.49*$^{++}$	16.19±3.64^{++}	32.37±2.97^{++}
Group VI	200 mg/kg LPHE+ $KBrO_3$	3.07±0.210^{++}	18.48±1.58^{++}	16.48±3.87^{++}	31.96±3.16^{++}

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.

Table 2. Effects of various fractions of *L. procumbens* on GST, GSH-Px, GSR, QR and LDH activity in liver of rat.

Group	Treatment	GST (nM/min/mg protein)	GSH-Px (nM/min/mg protein)	GSR (nM/min/mg protein)	QR (nM/min/mg protein)	LDH (nM/min /mgprotein)
Group I	Control	37.02±1.41^{++}	59.08±2.69^{++}	76.13±3.98^{++}	105.67±5.33^{++}	110.05±2.30^{++}
Group II	20 mg/kg $KBrO_3$	22.82±0.511**	32.07±1.00**	36.32±1.50**	52.35±2.00**	203.12±4.69**
Group III	200 mg/kg LPME+ $KBrO_3$	35.54±1.60^{++}	48.54±3.20^{++}	66.53±4.81^{++}	84.80±3.56**$^{++}$	118.67±2.58^{++}
Group IV	200 mg/kg LPCE+ $KBrO_3$	34.10±1.36^{++}	49.38±2.22^{++}	61.66±4.30**$^{++}$	86.35±3.08**$^{++}$	108.73±4.3^{++}
Group V	200 mg/kg LPEE+ $KBrO_3$	33.29±1.52^{++}	51.47±3.00^{++}	64.66±4.48**$^{++}$	90.40±4.46**$^{++}$	127.67±5.01^{++}
Group VI	200 mg/kg LPHE+ $KBrO_3$	34.63±1.59^{++}	58.11±3.17^{++}	74.59±4.74^{++}	93.65±3.04^{++}	139.98±3.87^{++}

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.

Table 3. Effect of various fractions of *L. procumbens* on TBARS, H_2O_2, GSH and nitrite contents in liver of rat

Group	Treatment	GSH (M/g tissue)	TBARS (nM /min/mg protein)	H_2O_2 (nM /min/mg tissue)	Nitrite (M/ml)
Group I	Control	0.47±0.04^{++}	28.631±0.118^{++}	5.61±0.118^{++}	46.78±2.62^{++}
Group II	20 mg/kg $KBrO_3$	0.18±0.0129**	49.2±0.107**	8.4±0.503**	76.70±2.43**
Group III	200 mg/kg LPME+ $KBrO_3$	0.43±0.035^{++}	34.9±0.088^{++}	6.08±0.509^{++}	53.12±2.40^{++}
Group IV	200 mg/kg LPCE+ $KBrO_3$	0.38±0.019^{++}	34.6±0.088^{++}	5.64±0.076^{++}	61.28±2.57**$^{++}$
Group V	200 mg/kg LPEE+ $KBrO_3$	0.448±0.0295^{++}	36.9±0.088^{++}	5.62±0.0824^{++}	66.12±3.07**$^{++}$
Group VI	200 mg/kg LPHE+ $KBrO_3$	0.4697±0.0437^{++}	33.9±0.088^{++}	5.92±0.098^{++}	65.2± 1.57**$^{++}$

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.

ROS into active chemicals but also magnifies the function of ROS through the chain reaction, inducing cellular metabolism and functional impairment (Khan et al., 2011), and serves to indicate the presence of free radicals, lipid peroxide formation. Effects of various fractions of *L. procumbens* on the content of GSH, TBARS, H_2O_2 and nitrite are shown in Table 3. Administration of $KBrO_3$ for 4 weeks significantly depleted (P<0.01) the GSH while elevated (P<0.01) the nitrite, TBARS and H_2O_2 contents than control. Post-administration of of 200 mg/kg b.w various fractions of *L. procumbens* in $KBrO_3$ treated rats significantly reduced (P<0.01) TBARS, H_2O_2 and tissue nitrite contents while increased significantly (P<0.01) the GSH contents by detoxifying the $KBrO_3$ oxidative stress in a dose dependent manner. Similar results are reported by Khan et al. (2009) and Sahreen et al. (2011).

Conclusion

From the data of various fractions of *L. procumbens*, it is

recommended as an herbal medicine for the treatment of liver oxidative stress.

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