Protective effects of *Sonchus asper* (L.) against $KBrO_3$-induced oxidative stress in rat testis

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Abstract: *Sonchus asper* is used traditionally in the treatment of kidney inflammation, hormonal imbalance and impotency. *Sonchus asper* methanolic extract (SAME) was investigated for its possible preventive effect against potassium bromate ($KBrO_3$) induced oxidative damages in male rats using biochemical, molecular and histopathological markers in this study. 5 groups, each group of 6 rats were taken kept under standard conditions. Group 1 remained untreated while Group II was given 20 mg/kg $KBrO_3$ orally (in aqueous saline) and Group III, and IV were treated with 100; 200 mg/kg b.w., of SAME after 48 h of $KBrO_3$ treatment. $KBrO_3$ administration in rats significantly altered *(P<0.01)* the serum level of reproductive hormones, activities of antioxidant enzymes and glutathione contents (GSH), which was significantly reversed *P<0.001)* by co-treatment of 100 mg/kg and 200 mg/kg b.w., SAME. Administration of SAME in rats also significantly *P<0.001)* reversed the lipid peroxidation induced by $KBrO_3$ in rats, which could be due to the presence of some plant bioactive constituents.

Keywords: $KBrO_3$, oxidative stress, antioxidant enzymes, TBARS contents, LH.

INTRODUCTION

Free radicals and reactive oxygen species are produced during respiration, metabolism and exposure of environmental toxins. Potassium bromate ($KBrO_3$) molecular weight 166 g/mol is an oxidizing agent, 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). Intake of $KBrO_3$ or exposure to it causes production of oxygen free species in living cells. Reactive oxygen species are highly reactive molecules that cause many degenerative diseases like Alzheimer disease, Parkinson's disease, multiple sclerosis, Down's syndrome, inflammation, and ulcer as well as degradation of biomolecules, protein degradation, depletion of antioxidant enzyme activity and lipid peroxidation of membrane, hormonal imbalance and DNA damages (Khan *et al.,* 2011). Medicinal plants play important role in the treatment of many degenerative disorders. To support endogenous antioxidant enzymatic system various antioxidant therapies are used. For thousands of years, medicinal plants are used as a source of medicine and to improve human life. Several herbs possess bioactive constituents such as phenolic and polyphenolic compounds which regulate various immune systems. The flavonoids and phenolic compounds rich herbs may also possess antioxidant and anti-inflammatory properties (Tyler, 1994; Bruneton, 1995). *Sonchus asper* (L.) locally named as Mahtari used in the treatment of liver injuries (Rivera and Oben, 1993), cancer (Thomson and Shaw, 2002), kidney inflammation (Khan *et al.,* 2010), pulmonary damages (Khan *et al.,* 2011) and cardiac dysfunction (Khan *et al.,* 2011). Therefore, here we aimed at studying the protective effects of *Sonchus asper* induced oxidative damages in rat testis.

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MATERIALS AND METHODS

Plant identification, collection and extraction

Various areas of Wah Cantt were searched out for collection of *Sonchus asper* after identification in the month of Aug 2010. A specimen was preserved in the Herbarium for future records. After dry whole plant (leaves, stem, flowers, roots and seeds) were ground with pounder and mortar. *Sonchus asper* powder was socked in methanol for a week and then filtered out. The filtrate was evaporated with help of rotary evaporator to collect methanolic extract.

Experimental design

30 male rats (190-200 g) were given by National Institute of Health (NIH) Islamabad, Pakistan and were placed at standard conditions. 5 groups (6 rats) were arranged to learn the protective effects of SAME. Group 1 received only raw water and free access to food materials. Group II received 20 mg/kg b.w., $KBrO_3$ orally. Groups III and IV received 100, 200 mg/kg body weight of SAME (intragastric, in DMSO) after 48 h of $KBrO_3$, respectively for four weeks. All animals were weighted after the last treatment and sacrificed. Testes were treated with liquid nitrogen to study the protective effects of SAME.

Assessment of serum markers

Serum analysis of various hormones such as testosterone, luteinizing hormones (LH) and follicle stimulating

hormones (FSH) was conducted by using standard diagnostic kits with Gamma counter.

Oxidative profile of antioxidant enzymes

70 mg testis was homogenized in 10 mmol phosphate buffer to get tissue supernatant with help of homogenizer. Soluble tissue protein was determined with the protocol of Lowry *et al.* (1951). Activities of Catalase, peroxidase (Chance and Maehly, 1955) and superoxide dismutase were estimated by using procedure of Kakkar *et al.* (1984). Activity of glutathione-*S*-transferase (Habig *et al.,* 1974), glutathione reductase activity (Carlberg and Mannervik, 1975), glutathione peroxidase (GSH-Px) (Mohandas *et al.,* 1984), quinone reductase (Benson *et al.,*1980), *Reduced glutathione assay (GSH)* (Jollow *et al.,* 1974), TBARS (Wright *et al.,*1981) as modified by Iqbal *et al.* (1996) and hydrogen peroxide (H_2O_2) was measured with protocol of (Pick and Keisari, 1981).

Nitrite assay

Supernatant of homogenate was collected after deproteinized with NaOH and $ZnSO_4$ and centrifugation at 6400 × g for 20 min. Griess reagent was used to blank the spectrophotometer at 540 nm and supernatant was added.

STATISTICAL ANALYSIS

SPSS ver. 14.0 (Chicago, IL, USA) and Microsoft Excel 2007 were used to analyse the mean and significance of data.

RESULTS

Effects of Sonchus asper on serum hormonal level

Protective effects of *Sonchus asper* on serum reproductive hormonal level are shown in table 1. Induction of $KBrO_3$ in rat significantly (P<0.01) altered the levels of testosterone, follicle stimulating hormone and luteinizing hormones. Co-treatment of 100 mg/kg b.w., and 200 mg/kg b.w., in $KBrO_3$ treated rats markedly (P<0.01) reversed the hormonal levels of testosterone, LH and FSH.

Effects of Sonchus asper on antioxidant enzymes

POD, CAT and SOD are a supportive team of antioxidant enzymes, and play an important role in detoxification of free radicals. Treatment of rats with $KBrO_3$ significantly (P<0.01) depleted the antioxidant enzymes activities including catalase, peroxidise and super oxide dismutase. Co-administration of *Sonchus asper* in rats significantly (P<0.01) protected the activities of antioxidant enzymes like CAT, POD and SOD which could be due the presence of bioactive constituents in the extract.

Effects of Sonchus asper on Phase II metabolizing enzymes

Phase II metabolizing enzymes include GSH-px, GST, GSR and QR activities. Administration of $KBrO_3$ in rats significantly (P<0.01) reduced the activities of glutathione reductase, glutathione peroxidise, glutathione-S-transferase and quinine reductase. Co-treatment of *Sonchus asper* in rats significantly improved the activities of these enzymes, proving that extract possess antioxidant constituents.

Effects of Sonchus asper on GSH, lipid peroxidation, H_2O_2 and nitrite contents

TBARS contents are the main constituents of lipid peroxidation. Administration of $KBrO_3$ in male albino rats are significantly (*P<0.01*) reduced GSH contents while enhanced TBARS and nitrite contents as compare to non treated control rats. Co-administration of 100 mg/kg b.w., and 200 mg/kg b.w., *Sonchus asper* methanolic extract in rats significantly (*P<0.01*) increased GSH contents while depleted TBARS and nitrite contents to that of control rat.

Table 1: Effect of *Sonchus asper* on serum male hormones in rat

Treatment	FSH (mg/dl)	LH (mg/dl)	Testosterone (mg/dl)
Control	22.0 ± 0.7++	24 ± 1.32++	45 ± 3.04++
20 mg/kg $KBrO_3$	11.2 ± 0.2**	12.5 ± 0.71**	24 ± 2.7**
100mg/kg SAME+$KBrO_3$	16.1 ± 0.98++	18 ± 1.43++	37 ± 1.21++
200mg/kg SAME+$KBrO_3$	18.3 ± 1.37++	22. ± 1.97++	42.5 ± 2.90++

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.

DISCUSSION

The present study focused on investigating the effect of SAME against $KBrO_3$-induced toxicity in male rats. Formerly, our research group had reported the protective effects of SAME against kidney inflammation (Khan *et al*., 2010) cardio toxicity (Khan *et al*., 2011) and pulmonary damages in rats (Khan *et al*., 2011). Our data revealed that reactive oxygen species cause alteration in the levels of reproductive hormones comparative to non treated control rats which were significantly (P<0.01) reversed by co-treatment of SAME in a dose dependent manner (table 1). Hypothalamus, pituitary and testis axis play important role in regulation of endocrine hormones and secrets gonadotropin (GnRH) i.e., FSH and LH (Conn, 1986).

Oxidative stress in rat testis induced by free radicals of potassium bromated causes alteration of testosterone levels due to injuries of Leydig cells (Santos *et al*., 2004). Depletion of testosterone further causes reduction of LH

Table 2: Effect of *Sonchus asper* on testis protein, CAT, POD and SOD activity in rat

Treatment	Protein (µg/mg tissue)	CAT (U/min)	POD (U/min)	SOD (U/mg protein)
Control	3.4±0.089++	7.19±1.18++	10.94±2.07++	20.69±2.97++
20 mg/kg $KBrO_3$	1.97.087**	4.151±0.54**	5.851±0.983**	13.55±1.43**
100mg/kg SAME+ $KBrO_3$	2.72±0.068**++	6.14±0.712*++	8.37±1.31**++	17.19±1.90*++
200mg/kg SAME+ $KBrO_3$	3.08±0.058++	6.421±0.841++	9.63±1.51++	19.84±2.17++

Table 3: Effect of *Sonchus asper* on testis GST, GSR, GSH-Px and QR activity in rat

Treatment	GSH-Px(nM/ mg protein)	GSR(nM/min/mg protein)	GST(nM/min/mg protein)	QR (nM/min/ mg protein)
Control	39.44±3.86++	63.44±3.86++	26.44±3.86++	43.14±6.82++
20 mg/kg $KBrO_3$	25.25±1.87**	44.25±1.87**	17.25±1.87**	26.80±3.30**
100mg/kg SAME+ $KBrO_3$	36.02±2.50++	54.02±2.50++	22.02±2.50++	36.21±4.40++
200mg/kg SAME+ $KBrO_3$	38.05±2.84++	58.05±2.84++	24.0±2.84++	39.88±5.02*++

Table 4: Effect of *Sonchus asper* on testis GSH, TBARS, H_2O_2 and nitrite contents in rat

Treatment	TBARS (nM/ min/mg protein)	H_2O_2 (nM/ min/mg tissue)	GSH (µM/g tissue)	Nitrite (µM/ml)
Control	19.78±1.18++	1.86±1.30++	0.875±0.0894++	45.43±3.66++
20 mg/kg $KBrO_3$	27.17±1.92**	5.58±2.11**	0.570±0.0443**	65.92±5.96**
100mg/kg SAME+$KBrO_3$	21.0±1.35++	3.09±1.49++	0.782±0.0595++	49.32±4.19++
200mg/kg SAME+ $KBrO_3$	20.07±1.23++	2.17±1.35++	0.858±0.0667++	46.31±3.80++

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.

and FSH leads to testicular dysfunction in male rats (Steinberger and Chowdhury, 1977). Co-treatment of rats with SAME significantly improved the alterations in the serum hormonal level as was induced by the treatment of $KBrO_3$ in rats. Antioxidant enzymes play important role in detoxification of free radicals (Khan *et al*., 2009; Khan and Ahmed, 2009). The main block of antioxidant enzymes are made of CAT, POD and SOD, which play key role in detoxification of oxygen free radicals (Sreelatha *et al.,* 2009). SOD catalyzes the superoxide anion into oxygen and hydrogen peroxide, which are later on converted into water by CAT and POD (Szymonik-Lesiuk *et al.,* 2003). Data of the present study revealed that SAME significantly protected the activities of antioxidant enzymes possibly due to the presence of bioactive constituents in the extract (Bingham *et al.,* 2003; Silva *et al.,* 2004). Manna *et al*. (2006) and Hassan *et al*. (2007) have obtained similar results.

Reactive oxygen free radicals react with glutathione causing reduction of glutathione contents and their related enzymes. Results of our present study revealed that free radicals of $KBrO_3$ cause depletion of GSH contents and depression of antioxidant enzyme activity of GSH-px, GSR and GST. Co-administration of SAME significantly reversed the activities of antioxidant enzymes.

Chemical induction in rats significantly decreased the GSH contents and increased lipid peroxidation. In our present study treatment of $KBrO_3$ depleted GSH contents while increased the lipid peroxide TBARS contents which are produced during the peroxidation of polyunsaturated fatty acids (PUFA) (Ohkawa *et al.,* 1979). Administration of different concentration of *Sonchus asper* considerably increased the GSH contents and depleted the lipid peroxide contents. Khan *et al*. (2009) and Sreelatha *et al*. (2009) reported comparable defensive effects by co-administration of *Digera muricata* and *Coriandrum sativum* extracts against oxidative stress against chemical induced toxicity in rats. $KBrO_3$ induction in rats caused constriction and damages of blood capillaries in rats that in turn leads to elevation of nitrite contents in tissue supernatant. Co-treatment of various concentrations of *Launaea procumbens* improved damages of blood capillaries. Similar studies have been obtained by (Khan *et al*., 2009; Khan, 2012).

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