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Full Length Research Paper

Prevention of $KBrO_3$-induced cardiotoxicity by *Sonchus asper* in rat

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The protective effects of *Sonchus asper* methanol extract (SAME) against $KBrO_3$-induced oxidative damage in heart tissues of rat were determined in this study. Sprague-Dawley male rats (180 to 200 g) were divided into five groups. Group I: received saline (1 ml/kg b.w., 0.85% NaCl) and DMSO (1 ml/kg b.w.); group II: were injected with $KBrO_3$ (20 mg/kg b.w., i.p.); group III and IV: treated with $KBrO_3$ and after 48 with SAME (100; 200 mg/kg b.w.) and group V: with SAME (200 mg/kg b.w.). All the treatments were given twice a week for four weeks. The results revealed that $KBrO_3$ induced oxidative stress as evidenced by the significant decrease in the activity levels of antioxidant enzymes (CAT, POD, SOD, GSH-Px, GSR, GST, QR, XO) and glutathione (GSH), while increase in the levels of γ-GT, MDA and H_2O_2 in cardiac samples compared with the control group. Serum levels of CK, CK-MB, LDH, uric acid, AST, ALT, ALP, triglycerides, total cholesterol, LDL, VLDL were elevated while HDL level was significantly reduced in $KBrO_3$ group compared with the control. Since SAME treatment reversed these responses dose dependently, it seems likely that *S. asper* methanol extract can protect the cardiac tissues against $KBrO_3$ mediated oxidative damage.

Key words: *Sonchus asper*, $KBrO_3$, oxidative stress, lipid peroxidation, CK-MB, LDH.

INTRODUCTION

A number of environmental contaminants, chemicals and drugs dramatically alter the structure and function of various tissues and produce multiple adverse effects in the liver, kidney, heart and intestine (Ozturk et al., 1997). A number of these compounds are consumed through food as food contaminants or food additives. Potassium bromate ($KBrO_3$), an oxidizing agent and food additive, is one of such compound and high levels of this contaminant have been shown to cause multiple adverse health effects in humans and laboratory animals implying a potential hazard. Of the several molecular mechanisms of cardiotoxicity that have been postulated, generation of reactive oxygen species (ROS) is believed to play a central role. Different oxidases of the heart, especially xanthine oxidase, monoamine oxidase, and NADH oxidase produce H_2O_2, which directly and/or indirectly through the production of highly reactive hydroxyl radicals can cause myocardial injury (Gupta and Singal, 1989; Vandeplassche et al., 1989). The myocardium has enzymatic and non-enzymatic systems to neutralize free radicals (Priscilla and Prince, 2009). But, due to its highly oxidative metabolism and fewer antioxidant defenses compared to other organs, heart is very sensitive to ROS induced damage (Saravanan and Pugalendi, 2006). Green leafy vegetables constitute a major part of any balanced diet and are good sources of minerals and vitamins. These beneficial effects of green leafy vegetables are attributed, at least in part to, antioxidants such as flavonoids, flavones, isoflavones, lignans, catechins and isocatechins.

S. asper (L.) Hill, “sow thistle” or puha, an edible wild member of the family Compositae, was chiefly used as green (Afolayan and Jimoh, 2008). In Pakistan, *S. asper* is used as an alternative in the treatment of wounds and burns (Rehman, 2006; Hussain et al., 2008; Qureshi et al., 2009), cough, bronchitis, asthma (Ahmad et al., 2006), malaria (Manan et al., 2007), gastrointestinal

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infections and inflammation, fungal infections, diabetic and cardiac dysfunction (Sabeen and Ahmad, 2009). Use of *S. asper* has also been reported in kidney and liver injuries, inflammations (Zabihullah et al., 2006) and in jaundice (Jan et al., 2009). *S. asper* contains comparable contents of phenol, flavonoids, flavonol and proantho-cyanidin (Balasundram et al., 2006). So, it is suggested to exhibit a wide range of physiological properties, such as antioxidant and free radical scavenging activity and especially cardioprotective effects. Therefore, we investigated the prophylactic effect of *S. asper* in rats subjected to $KBrO_3$- induced oxidative stress with reference to antioxidant enzymes and serum biochemical markers including lipid profile.

MATERIALS AND METHODS

Plant material

S. asper (L.) locally also named as "Mahtari" at maturity were collected from Islamabad (Pakistan) during spring, 2008. Plant was identified and a voucher specimen has been submitted vide accession # R-147 in the Herbarium of Pakistan, Quaid-i-Azam University Islamabad, Pakistan. Aerial parts of plant (leaves, stem and flowers) were shade dried at room temperature for two weeks, chopped, grinded and then milled mechanically to a fine powder of mesh size 1 mm.

Preparation of plant extract

Powdered plant material (1.50 kg) was extracted with 3.0 L of absolute methanol for 4 days. Residue was extracted twice and filtrate obtained was mixed and dried in rotary evaporator (Buchi Rotavapour, Switzerland) at 40°C.

Experimental design

Thirty six male Sprague Dawley rats weighing 180 to 200 g, were provided by the Animal House of National Institute of Health (NIH), Islamabad. The animals were maintained in an environmentally controlled room maintained at temperature 25±3ºC; humidity 54±5%; 12 h dark/light cycle. They had free access to standard laboratory rat chow and fresh water *ad libitum* at the Primate Facility, Quaid-i-Azam University, Islamabad. All animals received humane care according to the study protocol for the care and use of animals approved by the Animal Ethics Committee of Quaid-i-Azam University, Islamabad. Rats were randomly divided into six groups (n = 6) as follows: Group I: (Control) administered with 1 ml/kg b.w. of saline (0.85% NaCl) (i.p.) and after 48 h by DMSO (intragastric) twice a week for four weeks; group II: received $KBrO_3$ (1 ml/kg b.w., 20 mg/ml aqueous solution) twice a week for four weeks; group III: $KBrO_3$ (1 ml/kg b.w., 20 mg/ml aqueous solution) twice a week + after 48 h by SAME (100 mg/kg b.w.) twice a week for four weeks; group IV: $KBrO_3$ (1 ml/kg b.w., 20 mg/ml aqueous solution) twice a week + after 48 h by SAME (200 mg/kg b.w.) twice a week for four weeks; group V: SAME (200 mg/kg b.w.) twice a week for four weeks. The animals were sacrificed after completion of the experiment.

Biochemical analysis

Trunk blood samples were collected and serum obtained was stored at –20°C until for various biochemical analyses. Creatine kinase (CK) and Creatine kinase-MB Fraction (CK-MB) levels were assayed using the standard diagnostic kits (Bayer Diagnostics). Lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and uric acid were assayed by Vita lab Spectra-2, Merck using diagnostic kit (Diasys). Serum level of total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) and triglycerides were determined by AMP diagnostic kits.

Antioxidant enzymes

Heart tissue samples were homogenized in 10 volume of chilled 100 mM isotonic KH_2PO_4 buffer (0.1 M, pH 7.4) containing 1 mM EDTA (pH 7.4) for the determination of different antioxidant enzymes. The endogenous antioxidants, catalase (CAT) (Manna et al., 2006), peroxidase (POD) (Chance and Maehly, 1955); superoxide dismutases (SOD) (Kakkar et al., 1984); glutathione peroxidase (GSH-Px) (Mohandas et al., 1984); glutathione-*S*-transferase (GST) (Habig et al., 1974); glutathione reductase (GSR) (Carlberg and Mannervik, 1975); γ-glutamyl transpeptidase (γ-GT) (Orlowaski and Miester, 1973); xanthine oxidase (XO) (Athar et al., 1996) were estimated in a UV-VIS SmartSpeck spectrophotometer (BIORAD, Germany). The activity of quinone reductase (QR) was determined according to Benson et al. (1980).

Malondialdehyde (MDA), reduced glutathione (GSH) and hydrogen peroxide (H_2O_2) assays

The MDA levels, an end product of lipid peroxidation, were assayed by determining thiobarbituric acid-reactive substances (TBARS) according to Iqbal et al. (1996). Reduced glutathione (GSH) was estimated by the method of Jollow et al. (1974) by using 1,2-dithio-bis nitro benzoic acid (DTNB) as substrate. Hydrogen peroxide (H_2O_2) was assayed by H_2O_2-mediated horseradish peroxidase-dependent oxidation of phenol red by the method of Pick and Keisari (1981) based on the standard curve of H_2O_2 oxidized phenol red.

Statistical analysis

Data were expressed as mean and standard error of the mean (SM). Data were analyzed through one way analysis of variance (ANOVA) by computer software (SPSS 13.0.). A value of $p \leq 0.05$ was considered significant.

RESULTS

In the $KBrO_3$ treated group the activity level of antioxidant enzymes, CAT, POD and SOD in cardiac samples (Table 1) showed a significant ($P<0.001$) decrease as compared with the control group. The groups treated with $KBrO_3$ and SAME (100; 200 mg/kg b.w.) showed a significant ($P<0.001$) increase, in a dose dependent manner, in the activity level of CAT, POD and SOD compared with the $KBrO_3$ group. However, the activity levels of these antioxidant enzymes were statistically low from the control group. The SAME treated group did not exhibit antioxidant activity of CAT, POD and SOD that was different from the control group. As shown in Table 2 the activity levels of GSH-Px, GST and GSR were

Table 1. Effect of SAME on catalase, peroxidase and superoxide dismutase in heart of rat.

Group	Treatment	CAT (nM H_2O_2 consumed/min/mg of protein)	SOD (U/mg protein)	POD (U/min)
I	Saline + DMSO	163.52±6.36^{C}	53.39±5.54^{C}	16.68±3.53^{C}
II	$KBrO_3$	72.40±6.30^{c}	28.40±4.71^{c}	4.72±0.84^{c}
III	$KBrO_3$ + SAME (A)	116.50±5.56^{Cc}	35.61±5.59^{Ac}	8.85±2.85^{b}
IV	$KBrO_3$ + SAME (B)	135.41±6.20^{Cc}	46.54±5.64^{Ca}	12.34±4.44^{B}
V	SAME (B)	162.71±6.44^{C}	48.70±5.02^{C}	17.67±5.06^{C}

Mean ± SE (n = 6). Saline (1 ml/kg b.w., 0.85% NaCl), DMSO (1 ml/kg b.w.), $KBrO_3$ (20 mg/kg b.w.), SAME (A) (100 mg/kg b.w.), SAME (B) (200 mg/kg b.w.). ^{a}, ^{b}, ^{c} indicate significance at P<0.05, P<0.01 and P<0.001 from control group. ^{A}, ^{B}, ^{C} indicate significance at P<0.05, P<0.01 and P<0.001 from $KBrO_3$ group.

Table 2. Effect of SAME on GST, GSR and GSH-Px in heart of rat.

Group	Treatment	GST (nM CDNB conjugate formed/ min/mg protein)	GSR (nM NADPH oxidized/min/mg protein)	GSH-Px nM NADPH oxidized/min/mg protein)
I	Saline + DMSO	194.30±5.58^{A}	269.69±4.79^{A}	235.32±5.72^{A}
II	$KBrO_3$	82.70±6.18^{b}	134.39±5.68^{b}	142.38±6.53^{b}
III	$KBrO_3$ + SAME (A)	145.53±6.09^{Ab}	188.49±5.20$^{A\,b}$	171.29±4.93$^{A\,b}$
IV	$KBrO_3$ + SAME (B)	176.69±5.42$^{A\,b}$	228.51±5.32$^{A\,b}$	191.01±4.83$^{A\,b}$
V	SAME (B)	192.30±4.72^{A}	267.41±4.72^{A}	233.69±5.60^{A}

Mean ± SE (n = 6). Saline (1 ml/kg b.w., 0.85% NaCl), DMSO (1 ml/kg b.w.), $KBrO_3$ (20 mg/kg b.w.), SAME (A) (100 mg/kg b.w.), SAME (B) (200 mg/kg b.w.). ^{a}, ^{b} indicate significance at P<0.01 and P<0.001 from control group. ^{A}indicate significance at P<0.001 from $KBrO_3$ group.

Table 3. Effect of SAME on QR, γ-GT and XO in heart of rat.

Group	Treatment	QR (nmol of dichloroindophenol reduced/min/mg protein)	γ-GT (nM p-nitroaniline/min/mg protein)	XO (µg of uric acid of uric formed/min/mg protein)
I	Saline + DMSO	193.85±4.81^{A}	522.51±4.40^{A}	0.336±.066^{A}
II	$KBrO_3$	121.62±5.50^{a}	964.24±6.45^{a}	0.738±.063^{a}
III	$KBrO_3$ + SAME (A)	149.61±3.92^{Aa}	746.29±4.76^{Aa}	0.588±.042^{Aa}
IV	$KBrO_3$ + SAME (B)	172.67±6.05^{Aa}	612.50±5.21^{Aa}	0.397±.042^{A}
V	SAME (B)	191.29±4.73^{A}	519.38±3.34^{A}	0.333±.053^{A}

Mean ± SE (n = 6). Saline (1 ml/kg b.w., 0.85% NaCl), DMSO (1 ml/kg b.w.), $KBrO_3$ (20 mg/kg b.w.), SAME (A) (100 mg/kg b.w.), SAME (B) (200 mg/kg b.w.). ^{a}, indicate significance at P<0.001 from control group. ^{A}, indicate significance at P<0.001 from $KBrO_3$ group.

significantly ($p < 0.001$) lowered in $KBrO_3$ treated group in cardiac samples compared with controls. The groups administered both $KBrO_3$ and SAME (100, 200 mg/kg b.w.) had significantly ($p < 0.001$) enhanced the activity levels of GSH-Px, GST and GSR in cardiac samples compared with the group given $KBrO_3$ only. However, the increase in the activity level was more pronounced at higher dose of SAME (200 mg/kg b.w.). SAME administration alone did not significantly change the activity levels of GSH-Px, GST and GSR in cardiac samples when compared with those of the control group.

In the $KBrO_3$ treated group, activity levels of QR were decreased while of γ-GT and XO in cardiac samples were significantly ($p < 0.001$) increased when compared with the control group (Table 3). In the groups treated with both $KBrO_3$ and SAME (100; 200 mg/kg b.w.) the activity level of QR was increased whereas γ-GT and XO activity levels were significantly ($p < 0.001$) decreased as against the $KBrO_3$ group. The restoration of these enzymes was concentration dependent with SAME dosage. The SAME treated group did not show any statistical change as against the control group. The MDA level an indicator of

Table 4. Effect of SAME on MDA, GSH and H_2O_2 in heart of rat.

Group	Treatment	Lipid peroxidation (nmol MDA/mg protein)	GSH (nmol/g tissue)	H_2O_2 (nmol/g tissue)
I	Saline + DMSO	4.34 ± 0.89^{B}	0.467 ± 0.04^{B}	250.64 ± 5.57^{B}
II	$KBrO_3$	13.21 ± 1.09^{b}	0.335 ± 0.08^{b}	533.69 ± 5.84^{b}
III	$KBrO_3$ + SAME (A)	8.38 ± 1.28^{Bb}	0.374 ± 0.05^{Ab}	432.85 ± 5.36^{Bb}
IV	$KBrO_3$ + SAME (B)	6.63 ± 1.13^{Ba}	0.422 ± 0.05^{Bb}	382.41 ± 5.19^{Bb}
V	SAME (B)	4.48 ± 0.42^{B}	0.460 ± 0.06^{B}	252.41 ± 5.38^{B}

Mean ± SE (n = 6). Saline (1 ml/kg b.w., 0.85% NaCl), DMSO (1 ml/kg b.w.), $KBrO_3$ (20 mg/kg b.w.), SAME (A) (100 mg/kg b.w.), SAME (B) (200 mg/kg b.w.). [a], [b]indicate significance at P<0.01 and P<0.001 from control group. [A,B]indicate significance at P<0.01 and P<0.001 from $KBrO_3$ group.

Table 5. Effect of SAME on ALT, ALP and AST in serum of rat.

Group	Treatment	ALT (U/L)	ALP (U/L)	AST (U/L)
I	Saline + DMSO	31.80 ± 4.45^{A}	111.04 ± 11.80^{A}	76.69 ± 9.24^{A}
II	$KBrO_3$	62.41 ± 6.11^{a}	210.31 ± 3.12^{a}	175.39 ± 4.74^{a}
III	$KBrO_3$ + SAME (A)	49.40 ± 3.63^{Aa}	155.69 ± 6.20^{Aa}	145.40 ± 5.56^{Aa}
IV	$KBrO_3$ + SAME (B)	35.44 ± 5.63^{A}	134.60 ± 5.57^{Aa}	97.60 ± 4.65^{Aa}
V	SAME (B)	29.64 ± 4.00^{A}	116.70 ± 3.90^{A}	79.86 ± 3.29^{A}

Mean ± SE (n = 6). Saline (1 ml/kg b.w., 0.85% NaCl), DMSO (1 ml/kg b.w.), $KBrO_3$ (20 mg/kg b.w.), SAME (A) (100 mg/kg b.w.), SAME (B) (200 mg/kg b.w.). [a], indicate significance at P<0.001 from control group. [A]indicate significance at P<0.001 from $KBrO_3$ group.

Table 6. Effect of SAME on total cholesterol, HDL, LDL, VLDL and triglycerides in serum of rat.

Group	Treatment	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	Triglycerides (mg/dl)
I	Saline + DMSO	101.85 ± 5.30^{A}	47.54 ± 5.03^{A}	60.69 ± 6.08^{A}	35.85 ± 5.41^{A}	135.70 ± 5.86^{A}
II	$KBrO_3$	209.69 ± 4.78^{b}	33.41 ± 5.47^{b}	112.30 ± 6.36^{b}	63.69 ± 6.57^{b}	180.60 ± 4.74^{b}
III	$KBrO_3$ + SAME (A)	165.31 ± 3.65^{Ab}	36.71 ± 4.78^{Ab}	94.32 ± 6.57^{Ab}	57.70 ± 7.89^{Aa}	161.69 ± 6.68^{Ab}
IV	$KBrO_3$ + SAME (B)	125.15 ± 5.79^{Ab}	41.72 ± 7.07^{Aa}	87.60 ± 5.27^{Ab}	46.69 ± 4.77^{Ab}	159.69 ± 4.18^{Ab}
V	SAME (B)	102.31 ± 5.70^{A}	46.31 ± 5.61^{A}	62.31 ± 5.59^{A}	36.71 ± 6.30^{A}	134.31 ± 5.61^{A}

Mean ± SE (n = 6). Saline (1 ml/kg b.w., 0.85% NaCl), DMSO (1 ml/kg b.w.), $KBrO_3$ (20 mg/kg b.w.), SAME (A) (100 mg/kg b.w.), SAME (B) (200 mg/kg b.w.). [a], [b]indicate significance at P<0.01 and P<0.001 from control group. [A], indicate significance at P<0.001 from $KBrO_3$ group.

lipid peroxidation was significantly higher while the contents of GSH, an endogenous antioxidant were significantly ($p < 0.001$) lower in $KBrO_3$ group compared with the control group (Table 4).

The groups administered both with $KBrO_3$ and SAME (200 mg/kg b.w.) had significantly decreased MDA and significantly increased GSH levels ($p < 0.001$) in cardiac samples compared with the group given $KBrO_3$ only. SAME administration alone did not statistically change the MDA and GSH levels in the cardiac samples when compared with the controls. As shown in Table 5 treatment of rats with $KBrO_3$ significantly elevated ($p < 0.001$) the activities of AST, ALP and ALT in serum relative to control group. The groups exposed to $KBrO_3$ along with different dosages of SAME (100; 200 mg/kg b.w.) significantly ($p < 0.001$) alleviated the toxicity induced with $KBrO_3$ and the activity of AST, ALP and ALT, dose dependently, restored towards the control group. However, administration of SAME (200 mg/kg b.w.) alone did not change the levels of AST, ALT and ALP when compared with the control group. Treatment of $KBrO_3$ to rats significantly ($p < 0.001$) enhanced the lipid profile, total cholesterol, LDL, VLDL and triglycerides compared with the control group (Table 6). HDL contents were significantly reduced with $KBrO_3$ administration when compared with control. The groups treated with

Table 7. Effect of SAME on CK, CK-MB, LDH and uric acid in serum of rat.

Group	Treatment	CK (U/L)	CK-MB (U/L)	LDH (U/L)	Uric acid (mg/dl)
I	Saline + DMSO	253.70±7.90^{A}	356.33±11.09^{A}	34.35±7.90^{A}	3.40±0.89^{A}
II	$KBrO_3$	327.85±5.07^{a}	831.83±13.40^{a}	86.69±5.55^{a}	18.72±0.90^{a}
III	$KBrO_3$ + SAME (A)	289.48±4.01^{Aa}	651.00±10.58^{Aa}	60.68±6.28^{Aa}	13.69±0.94^{Aa}
IV	$KBrO_3$ + SAME (B)	272.50±8.87^{Aa}	469.50±14.78^{Aa}	47.83±4.65^{Aa}	9.71±0.92^{Aa}
V	SAME (B)	257.58±6.05^{A}	338.50±17.20^{A}	32.31±3.54^{A}	3.55±0.96^{Aa}

Mean ± SE (n = 6). Saline (1 ml/kg b.w., 0.85% NaCl), DMSO (1 ml/kg b.w.), $KBrO_3$ (20 mg/kg b.w.), SAME (A) (100 mg/kg b.w.), SAME (B) (200 mg/kg b.w.). ^{a}indicate significance at P<0.001 from control group. ^{A}indicate significance at P<0.001 from $KBrO_3$ group.

both $KBrO_3$ and different dosages of SAME (100; 200 mg/kg b.w.) efficiently ($p < 0.001$) reduced the levels of total cholesterol, LDL, VLDL and triglycerides whereas increased ($p < 0.001$) the HDL levels in serum of rats compared with the $KBrO_3$ group. Treatment of rats with SAME alone (200 mg/kg b.w.) did not cause any statistical variation in the levels of above parameters in serum when compared with the control group. The group treated with $KBrO_3$ alone caused a significant ($p < 0.001$) elevation in the serum levels of CK, CK-MB, LDH and uric acid compared with the control group (Table 7). The increase in the serum levels of CK, CK-MB, LDH and uric acid with administration of $KBrO_3$ was significantly ($p < 0.001$) reduced with co-administration of different dosages of SAME (100; 200 mg/kg b.w.) when compared with the $KBrO_3$ group only.

SAME (200 mg/kg b.w.) treatment alone did not induce significant changes in the serum levels of CK, CK-MB, LDH and uric acid compared with the control group.

DISCUSSION

In the present investigation chronic administration of $KBrO_3$ to rats was shown to cause oxidative stress in heart and this damage was associated with significantly lowered levels of antioxidant enzymes; CAT, POD, SOD, GSH-Px, GSR, GST and QR whereas significant increase was observed for γ-GT in cardiac samples. Hydrogen peroxide levels in the cardiac samples were also elevated with $KBrO_3$ administration to rats. Further, this damage was observed to be associated with significant increase in lipid peroxides and a significant reduction in glutathione levels. Elevated levels of LDH, CK-MB in serum and xanthine oxidase in heart samples indicated the severity of $KBrO_3$ to rats. On the other hand, administration of SAME ameliorated the toxicity of $KBrO_3$ and restoring the changed levels of antioxidant enzymes and different biochemical parameters towards the control levels. Thus it seems likely that $KBrO_3$ cause heart injuries via the generation of free radicals while the SAME alleviated the tissue damage through free radical scavenging activities by the presence of different polyphenolics and other compounds (Khan et al., 2010). In the present study, SAME ameliorated the $KBrO_3$-mediated inhibition of the activities of antioxidant enzymes; CAT, POD, SOD, GSH-Px, GSR, GST and QR in heart samples. Living tissues are endowed with innate antioxidant defense mechanisms. Generally, SOD diminishes the superoxide toxicity by converting it to hydrogen peroxide while CAT and GSH-Px decomposes hydrogen peroxide and protects the tissue from highly reactive hydroxyl radicals. Previous studies have reported that oxidative stress plays an important role in the pathophysiology of $KBrO_3$-mediated damage (Watanabe et al., 2004). Abundant levels of tannic acid, flavonoids, flavonols and proanthocyanidins (catechin) were reported in *S. asper* (Balasundram et al., 2006), and could therefore contribute to its antioxidant activity. In fact, the most common group of indices used to assess oxidative stress is that of peroxidation products of lipids.

The observed mitigation in the MDA level in the heart of rats following simultaneous administration of $KBrO_3$ and SAME suggests its tissue protective potential by preventing the initiation and propagation of the lipid peroxidation process by scavenging (trapping) free radicals produced by $KBrO_3$. The decreased level of malondialdehyde in heart tissues could be due to the enhanced activities of antioxidant enzymes; CAT, POD, SOD, GSH-Px, GST, GSR and QR (Khan et al., 2009, 2010), due to the scavenging effects of SAME. An increase in H_2O_2 production by $KBrO_3$ in this experiment, as one of the active oxygen species in the oxygen radical chain reaction, may not only be caused by its increased production but may also be aided by a decrease in catalase and glutathione peroxidase activities subsequent to an increase in H_2O_2 as well as cause myocardial dysfunction (Takemura et al., 1993). The expression of γ-glutamyl transpeptidase (γ-GT) has been used as marker of chemical toxicity. In previous studies, Khan and Sultana (2004) reported that soy isoflavones ameliorated $KBrO_3$-mediated enhancement in the level of H_2O_2 in kidneys. Thus, in the present study, administration of SAME appears to have brought about a remarkable inhibition in the level of H_2O_2 in the rats heart that had been injected with $KBrO_3$. The central role of

reduced GSH appears clear in intracellular endogenous antioxidant defenses as it is involved in all the lines of protection against reactive oxygen species. Glutathione can directly scavenge free radicals or act as a substrate for the enzymes such as GSH-Px and GST during the detoxification of hydrogen peroxide, lipid hydroperoxides and electrophilic compounds (Ursini et al., 1995) and activities of both GSH-Px and GST can eventually lower the level of total intracellular GSH (Argyrou and Blanchard, 2004). In cardiomyocytes, GSH redox cycle is particularly important because the catalase level is low (Doroshow et al., 1980). Thus, the reduced levels of GSH observed in the cardiac samples of rats (administered $KBrO_3$) are consistent with the results of other workers (Farombi et al., 2002; Khan and Sultana, 2004). Interestingly, in the present study, SAME treated rats had a mean GSH level that was very similar to that of control rats. Similar increase in the GSH levels have been reported following administration of antioxidant-rich compounds such as kolavorin, a biflavonoid from *Garcinia kola* seeds (Farombi et al., 2002) and soy isoflavones (Khan and Sultana, 2004) to rats that had previously received $KBrO_3$.

An approach for detection of cardiac injury involves measurements of the well known cardiac marker enzymes such as creatine kinase (CK), creatine kinase-MB fraction (CK-MB), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), uric acid and lipid profile serum. Serum CK activity is considered as a sensitive indicator at an early stage of myocardial ischemia, while elevated levels of LDH is roughly proportional to the extent of injury to myocardial tissues (Hansi et al., 2009). Due to disturbance in the integrity of cardiac cell membranes as a consequence of peroxidation these enzymes enter into the blood stream thus increasing their concentration in the serum (Zhou et al., 2008). This might be due to the damage caused to the sarcolemma by the β-agonist that has rendered it leaky (Zhou et al., 2008; Goyal et al., 2010). However, these enzymes are not restricted to cardiac muscle tissue and their activity increases in the serum in non-cardiac tissue injuries also. Alkaline phosphatase activity on endothelial cell surfaces is responsible, in part, for the conversion of adenosine nucleotides to adenosine, a potent vasodilator and anti-inflammatory mediator that can protect tissues from the damage that results from injury. An increase in the concentration of total cholesterol, LDL and VLDL cholesterol and a decrease in HDL cholesterol are associated with raised risk of myocardial damage. Uric acid is considered to be a risk factor in the development of myocardial infarction. In hypoxic tissue, ATP depletion occurs which leads to accumulation of hypoxanthine. When tissues are disturbed, the enzyme xanthine dehydrogenase is converted to xanthine oxidase by the oxidation of essential 'SH' groups. XO catalyzes the conversion of hypoxanthine to xanthine, uric acid and superoxide (Weir et al., 2003).

A marked reduction in the levels of creatine kinase (CK), creatine kinase-MB (CK-MB), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), uric acid and lipid profile parameters of heart damage caused by SAME groups shows that prophylaxis of the plant is effective in improving cardiac function in $KBrO_3$-treated group.

Conclusion

The present investigation is important in presenting data suggesting considerable promise for the *S. asper* as a protective agent in $KBrO_3$-induced damage on heart tissues of rat. It is suggested that the mechanism of action of SAME is likely through scavenging of free radicals. Thus, our data suggest that *S. asper* is a potent antioxidant agent and inhibits $KBrO_3$-induced cardiotoxicity and oxidative damage response in male Sprague Dawley rats.

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