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ORIGINAL ARTICLE

Taurine ameliorates potassium bromate-induced kidney damage in rats

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Abstract Potassium bromate ($KBrO_3$) is widely used as a food additive and is a major water disinfection by-product. Several studies have shown that it causes nephrotoxicity in humans and experimental animals. We have investigated the potential role of the sulfonic amino acid taurine in protecting the kidney from $KBrO_3$-induced damage in rats. Animals were randomly divided into four groups: control, $KBrO_3$ alone, taurine alone and taurine + $KBrO_3$. Administration of single oral dose of $KBrO_3$ alone caused nephrotoxicity as evident by elevated serum creatinine and urea levels. Renal lipid peroxidation and protein carbonyls were increased while total sulfhydryl groups and reduced glutathione levels were decreased suggesting the induction of oxidative stress. The enzymes of renal brush border membrane were inhibited and those of carbohydrate metabolism were altered. There was an increase in DNA damage and DNA–protein cross-linking. Treatment with taurine, prior to administration of $KBrO_3$, resulted in significant attenuation in all these parameters but the administration of taurine alone had no effect. Histological studies supported these biochemical results showing extensive renal damage in $KBrO_3$-treated animals and greatly reduced tissue injury in the taurine + $KBrO_3$ group. These results show that taurine is an effective chemoprotectant against bromate-induced renal damage and this amino acid could prove to be useful in attenuating the toxicity of this compound.

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Abbreviations

AO	Antioxidant
AP	Alkaline phosphatase
BBM	Brush border membrane
BBMV	BBM vesicles
DPC	DNA–protein cross-linking
FBP	Fructose 1,6-bisphosphatase
G6PD	Glucose 6-phosphate dehydrogenase
G6P	Glucose 6-phosphatase
GGT	γ-Glutamyl transferase
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Reduced glutathione
H_2O_2	Hydrogen peroxide
$KBrO_3$	Potassium bromate
LAP	Leucine aminopeptidase
LDH	Lactate dehydrogenase
LPO	Lipid peroxidation
ME	Malic enzyme
MDH	Malate dehydrogenase
$NADP^+$	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate reduced
OS	Oxidative stress
ROS	Reactive oxygen species
SH	Sulfhydryl
TCA	Tricarboxylic acid
TBARS	Thiobarbituric acid reactive substances
UN	Urea nitrogen

Introduction

Potassium bromate ($KBrO_3$) is a food additive that is extensively used as a maturing agent for flour and as a dough conditioner. Bromate is also the major by-product generated during ozonation of surface water and is frequently detected in tap, and even bottled, water (IARC 1986). $KBrO_3$ is used in cosmetics and is a component of permanent hair weaving solutions. Exposure to $KBrO_3$ results in nephrotoxicity since kidney is the primary target organ of this compound. Chronic administration of $KBrO_3$ causes renal cell carcinomas in rats, hamsters and mice and thyroid and mesothelioma tumors in rats (Kurokawa et al. 1990; Wolf et al. 1998). Due to its cross species carcinogenicity, bromate is considered a probable human carcinogen and a complete carcinogen in animals.

The toxic effects of $KBrO_3$ are attributed to its ability to induce oxidative stress (OS) leading to enhanced production of reactive oxygen species (ROS) which are important mediators of tissue injury (EPA 2001). The ROS are widely thought to be generated in the cell due to reduction of $KBrO_3$ to bromide by intracellular reductants. $KBrO_3$ has been shown to induce oxidative modification of lipids and proteins in several animal tissues (Chipman et al. 1998; EPA 2001; Kurokawa et al. 1990). Increased levels of intracellular ROS due to exposure to $KBrO_3$ also result in DNA damage causing mutations, rearrangements, and transcriptional errors that impair important cellular functions, in many cases leading to cell death (Ballmaier and Epe 1995; Umemura et al. 2009). Supporting the involvement of ROS in bromate action, several antioxidants (AO) have been shown to ameliorate the bromate-induced toxicity (El-Sokkary 2000; Karbownik et al. 2006; Khan et al. 2004, 2012; Nishioka et al. 2006; Sultan et al. 2012).

Taurine (2-aminoethane sulfonic acid, $NH_3^+–CH_2–CH_2–SO_3^{2-}$) is the major intracellular-free beta-amino acid and is one of the few known naturally occurring sulfonic acids. It is present in various foods like eggs, milk and is especially abundant in seafood and meat; it is found in high concentrations in the most animal tissues (Huxtable 1992). Taurine is also used as an ingredient in many energy drinks, many containing up to 1,000–2,000 mg per serving. Taurine has many fundamental biological roles such as conjugation of bile acids, antioxidation, osmoregulation, membrane stabilization and modulation of calcium signaling. This amino acid has been shown to be essential for the development and survival of mammalian cells, particularly those of the cerebellum, retina, and kidney (Huxtable 1992). Studies have shown that taurine and the taurine transporter play an important role in kidney development (Han et al. 2000). Taurine is known to be an AO and is a potent scavenger of the hydroxyl radical suggesting that it may be useful in treating oxygen radical pathophysiology (Ripps and Shen 2012). Taurine has shown promise in protecting tissues from various free radicals associated pathological conditions resulting from exposure to heavy metals, drugs and toxins (Waterfield et al. 1994; Timbrell et al. 1995; Sener et al. 2005; Tabassum et al. 2007; Manna et al. 2009; Das et al. 2012; Rashid et al. 2012).

We have previously shown that a single oral dose of $KBrO_3$ induces nephrotoxicity in rats and alters the renal redox and metabolic status (Ahmad et al. 2012b). In the present work, we have explored the use of taurine in attenuating bromate-induced kidney damage using rats as the animal model. This was done in view of the reported involvement of oxidative stress (OS) in the mode of action of bromate toxicity and the effectiveness of taurine in mitigating toxicities involving ROS and OS. Since OS stress is well known to induce DNA and membrane damage and also alter metabolic pathways (Imlay 2003; Bergamini et al. 2004; Halliwell 2007) these parameters were studied and correlated to the histological changes that occur in the kidney. Therefore, the aim of this work was to examine if taurine could attenuate the bromate-induced (1) nephrotoxicity (increase in serum urea nitrogen and creatinine levels), (2) OS, (3) changes in carbohydrate metabolism, (4) DNA damage, and (5) inhibition of BBM enzymes. The biochemical alterations induced by $KBrO_3$, in presence and absence of taurine, were then correlated to the ensuing histological changes in the kidney. Our results show for the first time the effectiveness of taurine in protecting animals from bromate-induced nephropathy and oxidative damage in renal tissue.

Materials and methods

Adult male rats, 10–12 weeks old, of Wistar strain weighing 150–200 g were used in all the experiments. They were purchased from the Central Animal House Facility of Jamia Hamdard University, New Delhi. Animal experiments were conducted according to the guidelines of Ministry of Environment and Forests, Government of India, approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) vide registration no. 714/02/a/CPCSEA. The study was approved by the Institutional Animals Ethic Committee (IAEC) that monitors the research involving animals. All animals were stabilized for 1 week prior to the experiment on standard pellet rat diet with free access to water. Solutions of taurine and $KBrO_3$ were prepared in drinking water and they were given orally (by gavage) to the animals. The animals were randomly divided into four groups with six rats in each group.

1. *Control* Animals were given suitable volume of drinking water by gavage.
2. *$KBrO_3$ alone* Animals were given a single dose of $KBrO_3$ at 100 mg/kg body weight.
3. *Taurine alone* Animals were given taurine at 100 mg/kg body weight/day for 5 days.
4. *Taurine + $KBrO_3$* Animals were first given taurine for 5 days at 100 mg/kg body weight/day. Then 6 h after the last dose of taurine they were given single dose of $KBrO_3$ at 100 mg/kg body weight.

The animals were killed 48 h after the above treatments under light ether anaesthesia. The experiment was planned such that all animals were killed on the same day. All animals had free access to water and food throughout the duration of the experiment.

The blood and kidneys were removed and used in further analyses.

Glucose, urea nitrogen and creatinine levels in plasma

Blood was collected in heparinised tubes by cardiac puncture and was centrifuged at 900×*g* for 10 min at 4 °C in a clinical centrifuge. The plasma (supernatant) was used for determining creatinine, glucose and urea nitrogen (UN) levels. Glucose was determined by *o*-toluidine method and UN by diacetyl monoxime using kits from Span Diagnostics, India. Creatinine levels were determined in deproteinized plasma samples using saturated picric acid (Levinson and MacFate 1969).

Preparation of homogenates and brush border membrane vesicles (BBMV)

The kidneys were decapsulated and kept in ice-cold 154 mM NaCl, 5 mM Tris–HEPES buffer, pH 7.5. The cortex and medulla were carefully separated using a sharp scalpel and homogenized separately in a glass Teflon homogenizor in 50 mM mannitol, 2 mM Tris–HCl buffer, pH 7.0, to get a 10 % (w/v) homogenate. The homogenates were diluted to 5 % with Tris–mannitol buffer followed by high-speed homogenization (20,000 revolutions per min) in an Ultra Turrex Kunkel homogenizor (Janke and Kunkel IKA-Labortechnik, Staufen, Germany). The cortical and medullary homogenates were divided into aliquots and quickly frozen until further analysis. The BBMV were prepared from whole cortical homogenates using the $MgCl_2$ precipitation method exactly as described by Khundmiri et al. (1997). The final membrane preparations were suspended in 300 mM mannitol, 5 mM Tris–HCl buffer, pH 7.5 and used immediately or kept frozen until further use. Protein concentration in homogenates and BBMV was determined by the method of Lowry et al. (1951).

Assay of BBM enzymes

The enzymes were assayed in cortical and medullary homogenates and also BBMV as described by Khundmiri et al. (1997). Briefly, the activity of alkaline phosphatase (AP) was determined at pH 10.5 using *p*-nitrophenyl phosphate as substrate, γ-glutamyl transferase (GGT) and leucine aminopeptidase (LAP) were assayed using γ-glutamyl *p*-nitroanilide and L-leucine *p*-nitroanilide as substrates, respectively, and maltase by the glucose oxidase–peroxidase method. The kinetic parameters K_M and V_{max} were determined by assaying the enzymes at different substrate concentrations in isolated BBMV.

TBARS, carbonyl content, GSH, total SH and H_2O_2 levels

Several parameters of OS were determined in cortical and medullary homogenates. Lipid peroxidation (LPO) was determined from thiobarbituric acid reactive substances (TBARS) level (Buege and Aust 1978). Protein carbonyl content was determined after reaction with 2,4-dinitrophenyl hydrazine (Levine et al. 1990). Total sulfhydryl (SH) groups and reduced glutathione (GSH) were determined from the yellow color produced after reaction with 5,5′-dithiobis-2-nitrobenzoic acid (Sedlak and Lindsay 1968). Hydrogen peroxide (H_2O_2) levels were determined using xylenol orange as color reagent in the presence of 100 mM sorbitol (Gay and Gebicki 2000).

Enzymes of carbohydrate metabolism

The activities of enzymes involving oxidation of NADH or reduction of $NADP^+$ were determined spectrophotometrically at 340 nm using molar extinction coefficient of $6.22 \times 10^3\ M^{-1}\ cm^{-1}$. Lactate dehydrogenase (LDH), hexokinase (HK), malate dehydrogenase (MDH), glucose 6-phosphate dehydrogenase (G6PD), malic enzyme (ME), glucose 6-phosphatase (G6P) and fructose 1,6-bisphosphatase (FBP) activities were assayed as described by Khundmiri et al. (2004).

Histology

The decapsulated kidneys were nicked slightly and stored in 10 % formalin. They were processed for paraffin embedding, cut into 10 µm sections, and stained with hematoxylin and eosin by routine procedure (Culling 1974). The slides were examined using a trinocular microscope (Olympus BX40, Japan) at 100× magnification.

DNA fragmentation and DNA–protein cross-linking assay

Quantitation of DNA fragmentation was done by the colorimetric diphenylamine assay (Burton 1956). The kidney homogenates were mixed with equal volume of buffer containing 20 mM Tris–HCl, 20 mM EDTA, 0.5 % Triton X-100, pH 7.5, and centrifuged at 15,000 rpm for 15 min at 4 °C to separate intact DNA in the pellet from fragmented/damaged DNA in the supernatant fraction. Perchloric acid (final concentration 0.5 M) was added to the pellet and supernatant samples were heated at 90 °C for 15 min and then centrifuged to remove precipitated proteins. The resulting supernatants, whether containing whole or fragmented DNA, were treated with 58.7 mM diphenylamine for 16–20 h at room temperature in dark and the absorbance was recorded at 600 nm. DNA fragmentation was expressed as the percentage of fragmented DNA to total DNA.

DNA–protein cross-linking (DPC) was determined by mixing the homogenates in buffer containing 2 % SDS, 20 mM Tris–HCl, 20 mM EDTA, pH 7.5 and heating at 65 °C for 10 min. Then, 1.5 ml homogenates were mixed with 0.5 ml of 0.2 M KCl, 20 mM Tris–HCl, pH 7.5, passed five times through a 21-gauge needle and centrifuged. The DNA–protein cross-links were then determined in the supernatants exactly as described by Zhitkovich and Costa (1992).

Comet assay

Immediately after sacrifice, a small section of kidney cortex was transferred into the Roswell Park Memorial Institute (RPMI) medium containing 1 mM EDTA and finely chopped into very small pieces. The solutions were sieved by muslin cloth into Petri dishes to collect the cell suspension. The Comet assay was then performed by electrophoresis in alkaline condition as described by Singh et al. (1988). After electrophoresis, the DNA was stained by ethidium bromide and visualized under a CX41 fluorescence microscope (Olympus, Japan). The comets were scored at a magnification of 100× and images of 50 cells (25 from each replicate slide) for each sample were scored. Tail moment (comet tail length in μm multiplied by the fraction of total DNA in the tail) was used to assess nuclear DNA damage and was automatically generated by the Komet 5.5 (USA) image analysis system.

Statistical analysis

All data are expressed as mean ± standard error of mean (SEM). Analysis of variance was used in combination with post hoc test (Dunnett's multiple comparison test) using GraphPad InStat 3.0. (USA) to evaluate the data by comparing the results of treatment groups to control group. All differences of $p < 0.05$ were considered significantly different. All experiments were done at least three times to document reproducibility.

Results

The protective effect of taurine on renal toxicity induced by single oral dose of $KBrO_3$ was examined. The doses of taurine (100 mg/kg/day for 5 days) and $KBrO_3$ (single dose of 100 mg/kg) administered to animals were those which have been previously used by us and other workers (Das et al. 2009; Manna et al. 2009; Ahmad et al. 2012a, b). The dose of $KBrO_3$ used in the study is toxic but not lethal to the animals and no rat died during the course of the experiments. Both taurine and $KBrO_3$ were given orally to the animals, rather than intraperitoneally or subcutaneously, to simulate real exposure and intake of these agents. Animals in all four groups were killed 48 h after the treatments and their kidneys were removed. This time interval was selected since our previous work has shown that $KBrO_3$-induced renal changes were maximum 48 h after its administration (Ahmad et al. 2012b). The effect of $KBrO_3$, alone and in combination with taurine, was determined on nephrotoxicity, parameters of OS, DNA damage as well as enzymes of BBM and carbohydrate metabolism.

Plasma parameters

The effect of taurine on $KBrO_3$-induced nephrotoxicity was evaluated from plasma levels of creatinine, glucose and UN. Significant alterations in creatinine (3-fold), UN (2.5-fold) and glucose (1.5-fold) levels were seen after treatment with $KBrO_3$ alone compared to the control group showing the induction of nephrotoxicity (Table 1). Administration of taurine alone did not have any effect on these parameters which were similar to control values. Administration of taurine to animals prior to $KBrO_3$ treatment resulted in significant attenuation in the $KBrO_3$-induced alterations in glucose, creatinine and UN levels.

BBM enzymes

The specific activities of BBM enzymes (AP, GGT, LAP and maltase) were assayed in renal homogenates and isolated BBMV prepared from animals in the four groups. Treatment of rats with $KBrO_3$ alone resulted in a significant decrease (35–65 %) in the activities of these marker enzymes in the homogenates of both cortex and medulla (Table 2). The magnitude of this decrease in enzyme activities was greater in the medulla than cortex (except LAP). Maltase was the enzyme most affected by treatment

Table 1 Effect of taurine and $KBrO_3$ on plasma creatinine, glucose and urea nitrogen levels in plasma

	Control	$KBrO_3$ alone	Taurine alone	Taurine + $KBrO_3$
Creatinine	0.38 ± 0.04	1.29 ± 0.13[a]	0.37 ± 0.03[c]	0.48 ± 0.05[b]
Urea nitrogen	10.78 ± 1.15	26.51 ± 1.89[a]	9.86 ± 0.74[c]	15.75 ± 0.97[b]
Glucose	150.93 ± 3.41	87.25 ± 2.24[a]	148.75 ± 3.17[c]	121.45 ± 2.84[b]

Results are mean ± SEM for six different preparations

Glucose, urea nitrogen and creatinine levels are in mg/100 ml

[a] Significantly different from control

[b] Significantly different from $KBrO_3$ alone group and also from control

[c] Significantly different from $KBrO_3$ alone group but not from control

Table 2 Effect of taurine and $KBrO_3$ on the activities of BBM enzymes in homogenates of renal cortex and medulla

	Control	$KBrO_3$ alone	Taurine alone	Taurine + $KBrO_3$
LAP				
Cortex	4.84 ± 0.25	2.79 ± 0.16[a]	4.91 ± 0.22[c]	3.56 ± 0.18[b]
Medulla	3.85 ± 0.17	2.44 ± 0.11[a]	3.97 ± 0.19[c]	2.94 ± 0.11[b]
AP				
Cortex	4.04 ± 0.21	2.21 ± 0.11[a]	4.11 ± 0.18[c]	3.01 ± 0.18[b]
Medulla	3.66 ± 0.18	1.87 ± 0.09[a]	3.75 ± 0.14[c]	3.02 ± 0.12[b]
GGT				
Cortex	3.10 ± 0.11	1.54 ± 0.09[a]	3.17 ± 0.12[c]	2.74 ± 0.19[b]
Medulla	2.49 ± 0.08	1.13 ± 0.07[a]	2.55 ± 0.12[c]	2.10 ± 0.14[b]
Maltase				
Cortex	21.17 ± 2.47	8.09 ± 1.52[a]	21.94 ± 1.89[c]	19.28 ± 2.06[c]
Medulla	14.10 ± 1.19	5.16 ± 0.68[a]	15.31 ± 1.68[c]	12.08 ± 1.70[c]

Results are mean ± SEM for six different preparations

Specific activities of enzymes are in μmol/mg protein/h

LAP leucine aminopeptidase, *AP* alkaline phosphatase, *GGT* γ-glutamyl transferase

[a] Significantly different from control

[b] Significantly different from $KBrO_3$ alone group and also from control

[c] Significantly different from $KBrO_3$ alone group but not from control

with $KBrO_3$, both in cortex and medulla. Pre-treatment with taurine resulted in significant amelioration in the $KBrO_3$-induced reduction in the activities of these BBM enzymes. The specific activities of these enzymes were then determined in the BBM vesicles isolated by the $MgCl_2$ precipitation procedure. The activities of all four enzymes declined significantly in the purified BBMV also (Table 3). Pre-treatment with taurine resulted in significant amelioration in the $KBrO_3$-induced reduction in the activities of these enzymes. Administration of taurine alone had no effect on the activities of any of the BBM enzymes which were not significantly different from the control group.

The kinetic parameters, K_M (Michaelis constant) and V_{max} (maximal velocity), of the enzymes were then determined in BBMV prepared from cortical homogenates of animals in the four groups. This was done by assaying the enzymes at different substrate concentrations and analyzing the data by double reciprocal ($1/v$ versus $1/[S]$) Lineweaver–Burk plots. Treatment with $KBrO_3$ led to significant lowering of V_{max} values of all BBM enzymes (Table 4). However, prior administration of taurine led to a significant recovery in the V_{max} values. As expected, treatment with taurine alone did not alter the V_{max} values of any of the four enzymes. The K_M values of the BBM enzymes were not altered by any of these treatments.

Enzymes of carbohydrate metabolism

The effect of treatment with $KBrO_3$ alone and after administration of taurine was determined on the activities of several enzymes of carbohydrate metabolism in

Table 3 Effect of taurine and $KBrO_3$ on the activities of enzymes in isolated BBMV

	Control	$KBrO_3$ alone	Taurine alone	Taurine + $KBrO_3$
LAP	46.25 ± 3.47	23.69 ± 1.57[a]	47.01 ± 3.58[c]	33.12 ± 2.01[b]
AP	37.63 ± 3.01	18.42 ± 1.19[a]	38.80 ± 2.45[c]	27.42 ± 1.41[b]
GGT	24.28 ± 1.25	11.23 ± 0.27[a]	24.89 ± 1.57[c]	19.99 ± 1.64[b]
Maltase	194.01 ± 6.25	78.94 ± 3.52[a]	196.86 ± 11.91[c]	174.52 ± 8.57[b]

Results are mean ± SEM for four different preparations

Specific activities of enzymes are in μmol/mg protein/h

LAP leucine aminopeptidase, *AP* alkaline phosphatase, *GGT* γ-glutamyl transferase

[a] Significantly different from control

[b] Significantly different from $KBrO_3$ alone group and also from control

[c] Significantly different from $KBrO_3$ alone group but not from control

Table 4 Effect of taurine and $KBrO_3$ on kinetic parameters of enzymes in isolated BBMV

Enzyme	K_M (mmol/l)	V_{max} (μmol/mg protein/h)
LAP		
Control	0.18 ± 0.004	18.87 ± 1.22
$KBrO_3$ alone	0.16 ± 0.002	9.37 ± 0.63[a]
Taurine alone	0.15 ± 0.001	20.12 ± 1.76[c]
Taurine + $KBrO_3$	0.21 ± 0.004	14.72 ± 1.12[b]
AP		
Control	34.48 ± 1.60	74.80 ± 3.35
$KBrO_3$ alone	31.25 ± 1.45	37.82 ± 1.79[a]
Taurine alone	32.41 ± 1.39	75.14 ± 2.31[c]
Taurine + $KBrO_3$	33.32 ± 1.74	52.78 ± 1.94[b]
GGT		
Control	2.04 ± 0.014	11.97 ± 0.98
$KBrO_3$ alone	2.02 ± 0.011	5.02 ± 0.19[a]
Taurine alone	1.96 ± 0.008	12.27 ± 1.12[c]
Taurine + $KBrO_3$	2.05 ± 0.011	10.74 ± 0.66[c]
Maltase		
Control	17.69 ± 1.48	291.86 ± 9.45
$KBrO_3$ alone	15.38 ± 0.98	181.12 ± 7.14[a]
Taurine alone	16.66 ± 0.75	293.14 ± 11.01[c]
Taurine + $KBrO_3$	16.39 ± 1.01	230.45 ± 10.74[b]

K_M and V_{max} were calculated from double reciprocal (1/v vs. 1/[S]) Lineweaver–Burk plots

Results are mean ± SEM for four different preparations

LAP leucine aminopeptidase, *AP* alkaline phosphatase, *GGT* γ-glutamyl transferase

[a] Significantly different from control

[b] Significantly different from $KBrO_3$ alone group and also from control

[c] Significantly different from $KBrO_3$ alone group but not from control

homogenates of renal cortex and medulla. These include HK and LDH (glycolysis), MDH (citric acid cycle), G6P and FBP (gluconeogenesis), G6PD (pentose phosphate pathway) and ME (NADPH generation). $KBrO_3$ treatment significantly increased the activities of HK and LDH while the activity of MDH was decreased (Table 5). Administration of $KBrO_3$ also decreased the activities of gluconeogenic enzymes, glucose 6-phosphatase and fructose 1,6-bisphosphatase in cortex and medulla. The effect of $KBrO_3$ was also determined on G6PD and ME that are the major source of NADPH which is needed in various anabolic reactions. Treatment with $KBrO_3$ alone significantly decreased G6PD but increased ME activity. However, in the taurine + $KBrO_3$ group, there was significant attenuation in the $KBrO_3$-induced alterations in the activities of all these metabolic enzymes. This suggests that taurine restores the metabolic pathways that were altered by exposure to $KBrO_3$. Administration of taurine alone did not significantly alter the activities of any of these enzymes.

Parameters of oxidative stress

AO status is a potential biomarker to determine the physiological state of the cell, tissue or organ. The ROS generated under conditions of OS cause increase in LPO, protein oxidation as well as oxidation of protein and non-protein (mainly GSH) sulfhydryl groups. Administration of $KBrO_3$ alone greatly enhanced both LPO and protein oxidation as reflected in elevated levels of TBARS (twofold) and protein carbonyls (threefold) compared to the control group. It also resulted in significant reduction in total SH and GSH content (Table 6). There was also a marked increase in level of H_2O_2, an ROS, in renal homogenates in $KBrO_3$-treated animals. However, these $KBrO_3$-induced changes were significantly attenuated by administration of taurine prior to treatment with $KBrO_3$. Treatment with taurine alone did not significantly alter any of these parameters. The results indicate marked protection by taurine against $KBrO_3$-induced OS in renal tissue.

Table 5 Effect of taurine and $KBrO_3$ on enzymes of carbohydrate metabolism in homogenates of renal cortex and medulla

	Control	$KBrO_3$ alone	Taurine alone	Taurine + $KBrO_3$
HK				
Cortex	7.89 ± 0.14	19.15 ± 1.62[a]	8.04 ± 0.51[c]	10.06 ± 0.57[b]
Medulla	6.42 ± 0.16	16.14 ± 0.74[a]	7.04 ± 0.24[c]	8.17 ± 0.23[b]
LDH				
Cortex	19.85 ± 1.26	58.07 ± 3.12[a]	20.54 ± 1.31[c]	24.63 ± 2.44[b]
Medulla	22.71 ± 2.15	50.04 ± 2.55[a]	23.87 ± 1.94[c]	27.14 ± 2.01[b]
MDH				
Cortex	15.02 ± 1.43	4.73 ± 0.45[a]	15.30 ± 1.33[c]	12.50 ± 0.97[b]
Medulla	13.21 ± 0.94	3.79 ± 0.29[a]	13.70 ± 1.01[c]	10.82 ± 0.84[b]
ME				
Cortex	1.82 ± 0.14	15.45 ± 1.86[a]	1.95 ± 0.22[c]	7.64 ± 0.42[b]
Medulla	3.16 ± 0.47	24.42 ± 1.94[a]	3.42 ± 0.61[c]	10.42 ± 0.8[b]
FBP				
Cortex	1.11 ± 0.09	0.22 ± 0.03[a]	1.15 ± 0.08[c]	0.90 ± 0.06[b]
Medulla	1.36 ± 0.11	0.71 ± 0.04[a]	1.47 ± 0.13[c]	1.15 ± 0.09[c]
G6P				
Cortex	0.44 ± 0.05	0.19 ± 0.01[a]	0.49 ± 0.04[c]	0.30 ± 0.02[b]
Medulla	0.61 ± 0.04	0.30 ± 0.02[a]	0.70 ± 0.04[c]	0.45 ± 0.03[b]
G6PD				
Cortex	14.89 ± 1.74	6.62 ± 0.38[a]	15.26 ± 1.43[c]	12.41 ± 1.85[c]
Medulla	12.18 ± 1.33	4.04 ± 0.18[a]	12.44 ± 1.22[c]	10.45 ± 1.05[c]

Results are mean ± SEM for six different preparations

Specific activities of LDH, MDH, ME and G6PD are in nmol/mg protein/minute while FBP and G6P are in μmol/mg protein/h

HK hexokinase, *LDH* lactate dehydrogenase, *MDH* malate dehydrogenase, *ME* malic enzyme, *FBP* fructose 1,6-bisphosphatase, *G6P* glucose 6-phosphatase, *G6PD* glucose 6-phosphate dehydrogenase

[a] Significantly different from control

[b] Significantly different from $KBrO_3$ alone group and also from control

[c] Significantly different from $KBrO_3$ alone group but not from control

Table 6 Effect of taurine and $KBrO_3$ on some parameters of oxidative stress in homogenates of renal cortex and medulla

	Control	$KBrO_3$ alone	Taurine alone	Taurine + $KBrO_3$
TBARS				
Cortex	68.85 ± 2.01	145.01 ± 6.12[a]	66.01 ± 2.86[c]	82.14 ± 3.97[b]
Medulla	47.76 ± 1.11	110.54 ± 3.85[a]	44.21 ± 1.59[c]	56.23 ± 2.14[b]
Carbonyl				
Cortex	90.15 ± 4.17	268.21 ± 10.01[a]	88.63 ± 3.11[c]	130.64 ± 6.51[b]
Medulla	62.23 ± 1.94	179.65 ± 7.63[a]	60.91 ± 1.91[c]	86.53 ± 2.14[b]
Total SH				
Cortex	17.15 ± 1.30	7.68 ± 0.24[a]	18.01 ± 1.73[c]	14.28 ± 1.97[b]
Medulla	13.63 ± 0.47	6.89 ± 0.21[a]	14.66 ± 0.86[c]	11.89 ± 0.84[c]
GSH				
Cortex	2.84 ± 0.11	0.93 ± 0.07[a]	2.96 ± 0.09[c]	1.96 ± 0.11[b]
Medulla	1.94 ± 0.07	0.78 ± 0.04[a]	2.05 ± 0.03[c]	1.38 ± 0.05[b]
H_2O_2				
Cortex	248.34 ± 10.14	701.28 ± 25.68[a]	244.01 ± 12.97[c]	386.55 ± 13.64[b]
Medulla	175.64 ± 7.14	351.47 ± 18.74[a]	172.96 ± 8.86[c]	234.67 ± 12.74[b]

Results are mean ± SEM for six different preparations

TBARS and H_2O_2 levels are in nmol/gram tissue while total SH and GSH are in μmol/gram tissue

TBARS thiobarbituric acid reactive substances, *SH* sulfhydryl, *GSH* glutathione, *H_2O_2* hydrogen peroxide

[a] Significantly different from control

[b] Significantly different from $KBrO_3$ alone group and also from control

[c] Significantly different from $KBrO_3$ alone group but not from control

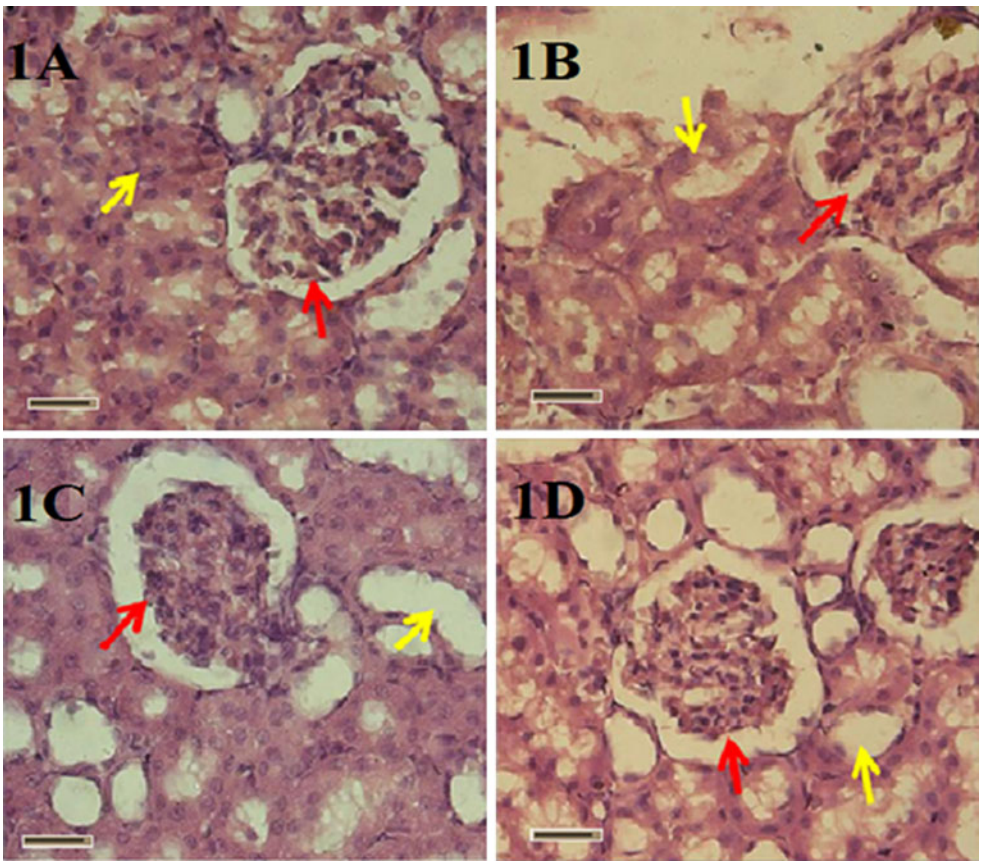

Fig. 1 Histology of hematoxylin- and eosin-stained sections of rat kidney showing glomerular capillary tuft (*red arrow*) in the renal corpuscle and renal tubule (*yellow arrow*). Kidney in the untreated control (**a**) shows normal corpuscle and tubular epithelium whereas $KBrO_3$-treated group (**b**) reveals extensive damage of both components. Kidney from taurine alone-treated animals (**c**) shows preserved conditions of both the components while taurine + $KBrO_3$ group (**d**) shows resumption in the morphology of renal corpuscle as well as renal tubule. ×400, *scale bar* [−] 50 μm

Histology

Histological examination of the kidney from control animals revealed normal histology including normal corpuscle and tubular epithelium (Fig. 1a). Marked histological changes were observed in kidneys in $KBrO_3$-treated rats, and extensive damage and renal injuries were visible (Fig. 1b). $KBrO_3$ treatment resulted in cellular swelling and tubular dilatation with damage to renal corpuscles and tubules. The taurine alone group showed reasonably well-preserved condition of both the components and thus taurine did not induce any histological lesions in the kidney (Fig. 1c). Pre-treatment with taurine greatly attenuated the toxic changes induced by $KBrO_3$ with the kidney showing much reduced damage (Fig. 1d).

DNA damage by Comet assay

The sensitive single cell gel electrophoresis assay (Comet assay), which is a simple and sensitive technique for the evaluation of DNA damage/repair and genotoxicity testing in mammalian cells, was used to study DNA damage in kidney. Administration of $KBrO_3$ to rats caused major DNA damage in renal tissue as evidenced by elongated tail moment with respect to the control. This indicates increased level of DNA single-strand breaks and alkali labile sites in the renal cells (Fig. 2). Significant DNA damage was observed in the renal cortex in $KBrO_3$-treated rats as compared to untreated control. Pre-treatment with taurine reduced the extent of $KBrO_3$-induced DNA damage as shown by decrease in tail moment. Taurine itself did not cause DNA damage in the renal tissue and the tail moment was almost the same as in control cells.

DNA fragmentation and DNA–protein cross-links

Oral administration of $KBrO_3$ led to significant induction of DPC both in renal cortex and medulla when compared to the untreated control group. The magnitude of DPC was much greater in cortex (354 %) as compared to medulla (163 %), respectively, compared to the control values (Table 7). Administration of $KBrO_3$ also induced DNA fragmentation

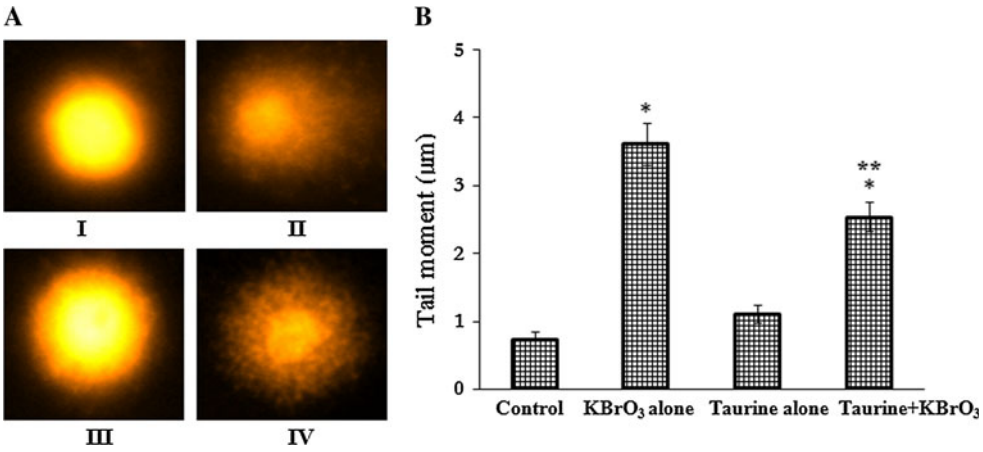

Fig. 2 **a** Comet assay. Renal cortical cells from control and all treated animals were subjected to alkaline single cell gel electrophoresis (comet assay) to assess the degree of DNA breakage. *I* Control, *II* $KBrO_3$ alone; *III* taurine alone, *IV* taurine + $KBrO_3$. **b** Tail moments. The results of comet assay (2A) are represented as mean values of tail moments. *Significantly different from control. **Significantly different from $KBrO_3$ alone group

Table 7 Effect of taurine and $KBrO_3$ on DPC formation in renal cortex and medulla

	Protein cross-linked DNA %*	DPC coefficient†
Control		
Cortex	3.13 ± 0.25	1
Medulla	1.92 ± 0.07	1
$KBrO_3$ alone		
Cortex	14.21 ± 0.75[a]	4.53
Medulla	5.06 ± 0.19[a]	2.64
Taurine alone		
Cortex	3.48 ± 0.18[c]	1.11
Medulla	2.11 ± 0.09[c]	1.09
Taurine + $KBrO_3$		
Cortex	9.17 ± 0.53[b]	2.93
Medulla	4.03 ± 0.37[b]	2.09

Results are mean ± SEM of six different preparations

DPC DNA–protein cross-links

* Protein cross-linked DNA/total DNA

† Protein cross-linked DNA (%) in treated animals/protein cross-linked DNA (%) in control animals

[a] Significantly different from control

[b] Significantly different from $KBrO_3$ alone group and also from control

[c] Significantly different from $KBrO_3$ alone group but not from control

with release of nucleotides in the supernatant as measured by the diphenylamine assay. The magnitude of DNA fragmentation was higher in cortex (167 %) than medulla (120 %). Pre-treatment with taurine significantly decreased $KBrO_3$-induced DNA fragmentation and DPC in renal tissues while taurine alone had no effect (Fig. 3).

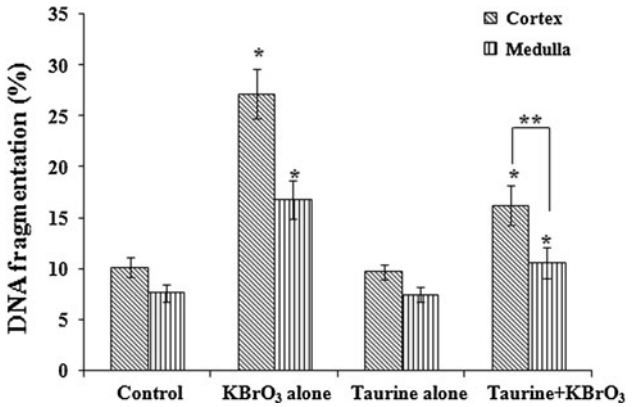

Fig. 3 Effect of pre-treatment with taurine on $KBrO_3$ induced DNA fragmentation in renal cortex and medulla determined by diphenylamine assay. Results are mean ± SEM of six different preparations. *Significantly different from control. **Significantly different from corresponding tissue (cortex or medulla) in $KBrO_3$ alone group

Discussion

Chemoprevention of cell, tissue and organ toxicity using naturally occurring dietary substances has gained increasing acceptance in recent years. Taurine is one such dietary compound which is found in several foods and has been shown to reduce the risk of various forms of cancer, cardiovascular and renal disorders and exhibits protective effects against certain environmental agents due to its AO, antimutagenic and anticarcinogenic properties (Alam et al. 2011; Das et al. 2012). In this study, we have used taurine as a potential protective agent against toxicity induced by $KBrO_3$, a well-known nephrotoxic compound that is used as a food additive and is also a water disinfection by-product. The amino acid taurine was selected since there are no reports of its use in mitigating toxicity of $KBrO_3$ or related oxyhalides. More importantly, the use of dietary compounds like taurine offers great potential since they are part of normal diet. Foods rich in this amino acid can be safely taken by persons who are at risk of exposure to such toxicants. A study by Clauson et al. (2008) found no adverse health effects of taurine used in energy drinks.

A single oral nephrotoxic dose of $KBrO_3$ was administered to animals with and without pre-treatment with

taurine. DNA damage/degradation and activities of various enzymes of carbohydrate metabolism, BBM and mitochondria were determined in renal tissue. Administration of $KBrO_3$ alone to rats produced a typical pattern of nephrotoxicity which was manifested by several fold increase in creatinine and UN levels, as also reported previously (Giri et al. 1999; Ahmad et al. 2012b). However, administration of taurine, prior to treatment with $KBrO_3$, led to significant recovery in both serum creatinine and urea nitrogen levels. Thus, pre-treatment of animals with taurine protects them from $KBrO_3$-induced nephrotoxicity.

The effect of $KBrO_3$ on BBM was also studied. BBM lines the epithelial cells and is the first barrier for various solutes during absorption in the kidney. It has been shown to be a major target of renal injury due to ischemia and nephrotoxic agents (Fatima et al. 2004; Goldman et al. 2006; Ahmad et al. 2012b). BBM and mitochondria are known to be targets of $KBrO_3$ (Ahlborn et al. 2009; Ahmad et al. 2012b); therefore, their structural/functional integrity was assessed from the activities of their respective biomarker enzymes. $KBrO_3$ caused significant decrease in the activities of BBM enzymes LAP, GGT, AP and maltase in renal homogenates and isolated BBM preparations, suggesting that the BBM was adversely affected by $KBrO_3$. The decrease in activities of BBM enzymes could be due to their loss/release from damaged BBM into the lumen of proximal tubule, as suggested for some nephrotoxicants (Kirschbaum and Oken 1979; Scherberich et al. 1993; Herak-Kramberger and Sabolic 2001; Basivireddy et al. 2005). Another possibility is the ROS-induced oxidative modification of amino acid side chains and consequent inactivation of the BBM enzymes (Dudeja and Brasitus 1993; Gupta et al. 1996; Nath and Norby 2000). Administration of taurine resulted in attenuation of $KBrO_3$-induced alterations in the activities of all these enzymes. Kinetic studies, using purified BBM vesicles, showed that the decrease in activities of these enzymes was due to change in V_{max} values while the K_M remained the same. Thus, the affinity of BBM enzymes for their substrates remains the same upon administration of taurine, $KBrO_3$ or taurine plus $KBrO_3$.

The reabsorption and transport of various ions and metabolites by renal BBM are considered to be the major function of the kidney. However, these transport processes depend on the structural integrity of BBM and available energy as ATP which is supplied by various metabolic pathways. Major alterations in these metabolic pathways caused by toxic agents would undermine the overall health of the tissue (Simpson 1983; Khundmiri et al. 2004). The activities of enzymes of different pathways of carbohydrate metabolism were, therefore, determined in homogenates prepared from animals in the four groups. The activities of enzymes involved in glycolysis (HK, LDH), TCA cycle (MDH), gluconeogenesis (G6P, FBP) and HMP shunt pathway (G6PD) were significantly altered upon $KBrO_3$ treatment. There was a significant increase in activities of LDH and HK with concomitant decrease in MDH in renal cortex and medulla upon $KBrO_3$ treatment. It also affected the enzymes of gluconeogenesis and HMP-shunt pathway differently. The activities of G6P and FBP were significantly decreased, whereas G6PD was profoundly increased in the renal cortex and medulla upon administration of $KBrO_3$. Although the actual rates of glycolysis or TCA cycle were not determined, marked decrease in MDH activity could be due to $KBrO_3$-induced damage to mitochondria. A marked increase in the activities of glycolytic enzymes, LDH and HK, appears to be an adaptive cellular response in energy metabolism from aerobic to anaerobic mode due to $KBrO_3$-induced mitochondrial dysfunction. This was accompanied by reduced synthesis of glucose. Taurine administration before treatment with $KBrO_3$ resulted in overall improvement of carbohydrate metabolism as evident by greatly restored activities of LDH, MDH and gluconeogenic enzymes. Thus, the $KBrO_3$-induced changes in renal carbohydrate metabolism are greatly restored towards normal by giving taurine to the animals.

ROS generated by various toxicants are important mediators of cellular injury and pathogenesis of various diseases. Various reports have shown that OS and ROS are important mediators of $KBrO_3$ nephropathy (Khan et al. 2004; Zhang et al. 2011). The higher levels of ROS increase oxidative modification of cellular components (Khan et al. 2004; Ahmad et al. 2012b), e.g. proteins, lipids and nucleic acids and damage the plasma membrane, mitochondria, microsomes and lysosomes in the kidney and other tissues (Giri et al. 1999; Oloyede and Sunmonu 2009; Ahmad et al. 2012b). In agreement with previous results (Ahmad et al. 2012b), $KBrO_3$ significantly decreased total thiols and enhanced LPO and protein carbonyls in renal tissues suggesting the induction of OS. The levels of H_2O_2, an ROS, were also significantly increased. H_2O_2 is a strong oxidant which can also generate the damaging hydroxyl radical upon reaction with transition metal ions in the cells. The levels of GSH, the most abundant low molecular weight thiol which acts as a physiological AO in the cell, were decreased. However, prior administration of taurine greatly lowered the $KBrO_3$-induced OS as evident by significantly recovered values of LPO and carbonyls along with total thiol levels. H_2O_2 and GSH levels were greatly restored to normal values compared to those in $KBrO_3$ alone-treated rats.

Free radicals can also damage DNA resulting in strand breakage and base modifications consequently leading to carcinogenesis (Waris and Ahsan 2006). Oral administration of $KBrO_3$ induced DNA fragmentation, as monitored by release of nucleotides from damaged DNA, both in renal

cortex and medulla when compared to control. This was confirmed by the Comet assay which showed increased level of DNA single-strand breaks and alkali labile sites in the kidney. DPC, which impedes the activities of proteins involved in DNA replication, transcription and repair, was also significantly enhanced in rats treated with $KBrO_3$. This is the first study showing enhanced formation of DPC in kidney in response to $KBrO_3$ treatment. The pre-treatment of animals with taurine reduced the level of $KBrO_3$-induced DNA damage and DPC formation probably by its anti-oxidant property, as reported in other systems also (Eppler and Dawson 2002; Saad and Al-Rikabi 2002; Tabassum et al. 2007).

Histological observations of the kidney strongly support the biochemical results. The kidneys from $KBrO_3$-treated animals showed extensive ultrastructural damage, with the renal tubule and the glomeruli being severely affected. These changes were greatly reduced by prior administration of taurine to the animals.

Our results clearly show that taurine can effectively reduce the $KBrO_3$-induced toxicity in the kidney. A similar protective effect of taurine against oxidative damage induced by various drugs/toxicants has been seen previously by other workers also (Eppler and Dawson 2002; Saad and Al-Rikabi 2002; Sener et al. 2005; Tabassum et al. 2007; Manna et al. 2009; Das et al. 2012). The protection seen here is probably due to the reduction in $KBrO_3$-generated OS by the AO property of taurine. Unlike polyphenolic antioxidants, taurine does not directly quench classical ROS and free radicals like H_2O_2, superoxide anion and hydroxyl radical due to its simple structure. However, taurine is widely believed to act as an indirect AO for which several reasons have been proposed (Roysommuti et al. 2003; Schaffer et al. 2003; Schuller-Levis and Park 2003; Atmaca 2004). First, taurine regulates the levels of various endogenous AO. It enhances the synthesis of GSH and also stimulates the activity of G6PD, an enzyme that generates NADPH which is required by glutathione reductase to convert oxidised glutathione into GSH. Second, taurine protects cells from necrosis by preventing Ca^{2+} overload via Na^{2+}/Ca^{2+} exchanger. Third, it stabilises the lipid bilayer and makes the membranes lipids less vulnerable to LPO. Fourth, due to its strongly negatively charged sulfonic group, taurine can conjugate redox active metals like Fe^{2+} and Cu^{2+}, thereby reducing their reaction with H_2O_2, a process which generates the damaging hydroxyl radical. Fifth, taurine has been shown to prevent the diversion of electrons from respiratory chain to other acceptors such as oxygen, thereby inhibiting the accumulation of ROS like superoxide anion. Finally, taurine can act as an organic osmolite by increasing tissue osmolarity and membrane flexibility. One or more of these mechanisms may be involved in protecting the kidney from toxicity induced by $KBrO_3$.

The above results have been summarized in Fig. 4. $KBrO_3$ enters the cell where it is reduced to bromide by intracellular reductants. This process is known to generate free radicals and ROS which then mediate tissue damage by oxidative modification of cellular components. Taurine either stops this reduction process or indirectly quenches/

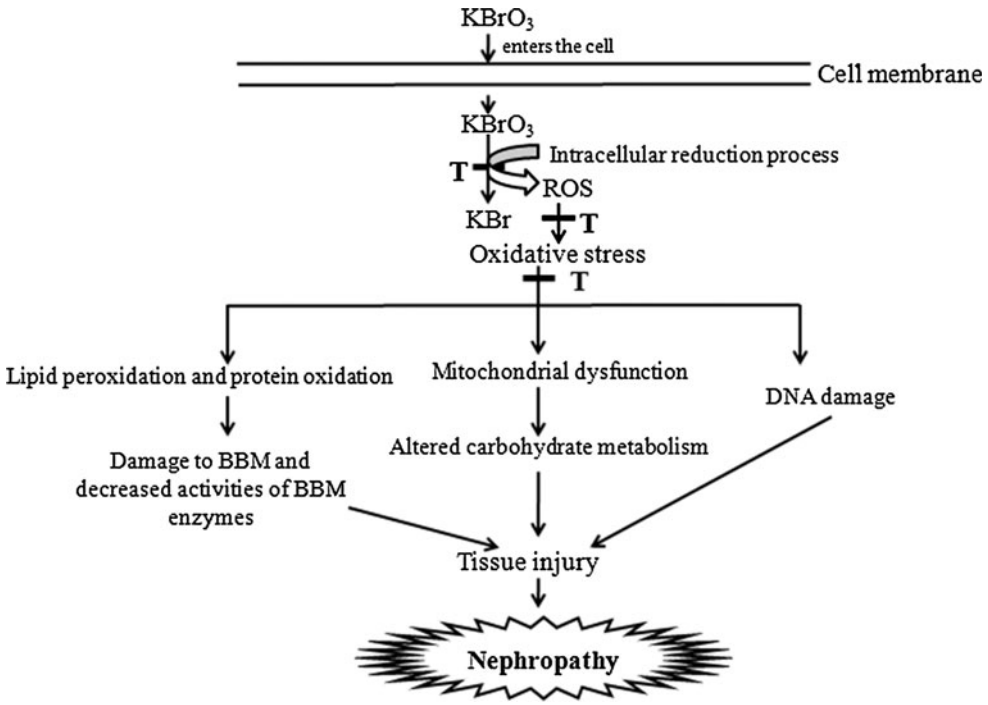

Fig. 4 $KBrO_3$ induced alterations in renal cell metabolism, membrane integrity and oxidative stress and its amelioration by taurine: a summary. *BBM* brush border membrane, *KBr* potassium bromide, *$KBrO_3$* potassium bromated, *ROS* reactive oxygen species, *T* taurine

scavenges the free radicals and ROS, thereby reducing oxidative damage to the cell components and restoring normal tissue morphology.

In conclusion, our results show that the sulfonic amino acid taurine protects the rat kidney from the extensive damage caused by oral administration of $KBrO_3$. Since $KBrO_3$ induces OS in the cells, which are thought to be responsible for its toxicity, the protective role of taurine may be due to its well-known ability to act as an AO and scavenger of ROS. Thus, taurine can be considered as a potential nephroprotective agent against renal damage caused by $KBrO_3$ and other structurally related compounds.

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Conflict of interest The authors declare there is no conflict of interest in this work.

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