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Prophylactic role of taurine on arsenic mediated oxidative renal dysfunction via MAPKs/ NF-κB and mitochondria dependent pathways

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Abstract

The present study has been designed and carried out to investigate the protective role of taurine (2-aminoethanesulphonic acid) against $NaAsO_2$ induced nephrotoxicity. Oral administration of arsenic increased the productions of ROS and RNS, enhanced lipid peroxidation, protein carbonylation and decreased intracellular antioxidant defence in the kidney tissue. Investigating the responsible signalling cascades, it was found that $NaAsO_2$ administration activates mitogen-activated protein kinases (MAPKs) and NF-κB in oxidative stress mediated renal dysfunction and induced apoptotic cell death by the reciprocal regulation of Bcl-2/Bad in association with reducing mitochondrial membrane potential and increased cytosolic cytochrome C as well. Treatment with taurine prior to arsenic administration effectively ameliorated As-induced oxidative renal dysfunctions and apoptotic cell death. Histological studies also support the experimental findings. Combining, results suggest that taurine possesses the ability to ameliorate arsenic-induced oxidative insult and renal damage, probably due to its antioxidant activity and functioning via MAPKs/NF-κB and mitochondria dependent pathways.

Keywords: *$NaAsO_2$, oxidative stress, reactive oxygen species, renal dysfunction, MAPKs/NF-κB and mitochondria dependent pathways, apoptosis, taurine, antioxidant, renal protection*

Abbreviations: *CAT, catalase; ERK, extracellular signal regulated protein kinases; FBS, Foetal bovine serum; FRAP, Ferric Reducing/Antioxidant Power; GSH, glutathione; GSSG, glutathione disulphide; GST, glutathione S-transferase; GPx, glutathione peroxidase; GR, glutathione reductase; HSD, hydroxysteroid dehydrogenase; MDA, malonaldehyde; MAPKs, mitogen-activated protein kinases; NF-κB, nuclear factor kappa B; ROS, reactive oxygen species; $NaAsO_2$, sodium arsenite; SOD, superoxide dismutase; TNF-α, tumour necrosis factor α*

Introduction

Arsenic (As) is widely used in agriculture, cosmetic industries, painting, wood preservation, semiconductors manufacturing, etc. [1]. However, it is also a potent environmental toxin. Elementary arsenic is fairly insoluble, whereas arsenic compounds dissolve readily in water. Its exposure occurs from inhalation, absorption through the skin and, primarily, by ingestion of contaminated food and drinking water [2]. Once absorbed, it can redistribute and induce dysfunctions in all the major organs of the body [3]. Kidney, the most important excretory and osmoregulatory organ, playing an important role in controlling and regulating homeostasis with reabsorptive, secretory and metabolic functions [4], is affected by As. Steps involving As poisoning and renal toxicity lack understanding, because absorbed toxin undergoes biomethylation to form monomethylarsinic and dimethylarsinic acids and are excreted by the kidney in the urine [5]. Since As generates reactive oxygen species (ROS) during metabolic activation processes, oxidative stress may be one of the mechanisms for arsenic-induced nephrotoxicity. Antioxidants have been found beneficial to mitigate chemical-induced

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and oxidative damage mediated organ pathophysiology [6,7]. Taurine (2-aminoethanesulphonic acid), a conditionally essential amino acid and a derivative of cysteine, has been considered as an antioxidant [8,9] and could be a potent candidate in this regard. It is present in many tissues in high concentrations. A number of investigators reported that taurine protects many of the body's organs against toxicity and oxidative stress induced by heavy metals and other toxins as well as drugs [10–13]. Although biochemical and physiologic functions of taurine are still undefined, considerable evidence shows that it can act as a direct antioxidant by scavenging ROS or as an indirect antioxidant by preventing changes in membrane permeability due to oxidative impairment [14,15].

The aim of the present study was, therefore, to investigate the prophylactic action of taurine against As-induced renal oxidative impairment and cell death. Distribution of arsenic in kidney tissues has been measured by atomic absorption spectroscopy. The *in vivo* antioxidant power of taurine in renal tissue was determined by Ferric Reducing/Antioxidant Power (FRAP) assay. The extent of renal damages caused by arsenic and the protective role of taurine was evaluated by measuring the (a) kidney weight to body weight ratios of experimental animals; (b) activities of serum marker enzymes related to renal dysfunction; (c) levels of cellular metabolites; (d) activities of intracellular antioxidant enzymes; (e) lipid peroxidation and protein carbonylation and (f) intracellular concentration of ROS.

Induction of nephro-pathophysiology was investigated by monitoring glomerular area, glomerular volume and the levels of blood urea nitrogen (BUN) as well as serum creatinine. Histological studies were carried out to assess the ultrastructural changes in the kidney tissue of the experimental animals. Molecular mechanism underlying As-induced renal dysfunction and protective effect of taurine was assessed by determining the DNA ladder formation, the role of mitogen-activated protein kinases (ERK 1/2, p38), NF-κB (p65) and Bcl-2 family proteins (Bcl-2, Bad). In addition, anti-apoptotic action of taurine was evaluated by measuring the mitochondrial membrane potential and cytosolic cytochrome C.

Materials and methods

Animals

Male albino rats of Wistar strain weighing $\sim$120–130 g (12 weeks old) were purchased from M/S Ghosh Enterprises (Kolkota, India). The animals were acclimatized under laboratory condition for 2 weeks prior to experiments. They were maintained under standard conditions of temperature ($25 \pm 1^{\circ}$C) and humidity (30%) with an alternating 12 h light/dark cycles. The animals were fed on standard diet and water *ad libitum*. All the studies were performed in conformity with the guidance for care and standard experimental animals study ethical protocols.

Chemicals

Kits for creatinine and urea nitrogen (UN) measurements were purchased from Span Diagnostic Ltd. (India). Sodium arsenite ($NaAsO_2$) and all other necessary reagents of analytical grade were bought from Sisco Research Laboratory (India). Bovine serum albumin (BSA), Bradford reagent, taurine (2-aminoethane sulphonic acid), ERK1/2 and phosphorylated ERK1/2 antibodies were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). NF-κB (p65 sub-unit), anti phosphorylated p65, p38 and phosphorylated p38 antibodies were purchased from Cell Signaling Technology (Beverly, MA). All other antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Determination of dose and time-dependent effect of arsenic

In this study, dose- and time-dependent toxic effects of $NaAsO_2$ were determined by measuring the SOD activity. For dose-dependent study, animals (12 weeks old) were randomly distributed into seven groups consisting of six animals in each. The first group served as normal control (received only water as vehicle). The remaining six groups were treated with six different doses of $NaAsO_2$ orally (0.5, 1, 2, 3, 4 and 5 mg/kg body weight for 2 weeks). For the determination of the time-dependent toxic effect, experiments were carried out with five groups of animals consisting of six in each group. The first group received water as vehicle and served as normal control. $NaAsO_2$ was administered orally to the other four groups at a dose of 3 mg/kg body weight for 0.5, 1, 2 and 3 weeks, respectively.

Twenty-four hours after the final dose of $NaAsO_2$ administration, all the animals were sacrificed, kidneys were collected and SOD assay were performed with kidney tissue homogenate.

Determination of dose- and time-dependent activity of taurine

Ferric Reducing/Antioxidant Power (FRAP) assay was performed to determine the optimum dose and time taurine needs for the protection of rodent kidneys against As-induced oxidative impairment. For this purpose, rats (12 weeks old) were divided into eight groups each consisting of six animals. The first two groups served as normal control (received only water as vehicle) and toxin control (received $NaAsO_2$ at a dose of 5 mg/kg body weight for 14 days, orally), respectively. The remaining six groups of animals were treated with six different doses of

taurine (10, 25, 50, 75, 100 and 150 mg/kg body weight for 5 days, orally) followed by $NaAsO_2$ intoxication (3 mg/kg body weight for 2 weeks, orally, once daily).

To determine the time-dependent effects of taurine in $NaAsO_2$-dependent renal disorder, rats (12 weeks old) were divided into nine groups each consisting of six animals. The first two groups served as normal control (received only water as vehicle) and toxin control (received $NaAsO_2$ at a dose of 3 mg/kg body weight for 2 weeks, orally), respectively. The other seven groups of animals were treated with taurine orally at a dose of 100 mg/kg body weight, once daily for 1, 2, 3, 4, 5, 6 and 7 days prior to $NaAsO_2$ intoxication (3 mg/kg body weight for 2 weeks, orally, once daily).

Twenty-four hours after the final dose of $NaAsO_2$ intoxication, all the animals were sacrificed, kidneys were collected and FRAP assays were performed with the kidney tissue homogenates.

Experimental design

The animals were divided into four groups, consisted of six rats (12 weeks old) in each and they were treated as follows:

- *Group 1*: Normal control (animals received only water as vehicle).
- *Group 2*: Toxin control (animals received $NaAsO_2$ orally at a dose of 3 mg/kg body weight for 2 weeks, once daily).
- *Group 3*: Animals were treated with taurine (orally, at a dose of 100 mg/kg body weight, once daily) for 5 days.
- *Group 4*: Animals were treated with a single dose of taurine (orally, at a dose of 100 mg/kg body weight, once daily) for 5 days followed by $NaAsO_2$ intoxication (orally, 3 mg/kg body weight, for 2 weeks once daily).

After the final dose of $NaAsO_2$, all the animals were sacrificed under light ether anaesthesia and kidneys were collected.

Estimation of renal arsenic content

The arsenic contents in renal tissues of all experimental animals (12 weeks old, four groups, consisted of six rats in each) were analysed following the method of Pari et al. [16] with some modifications. Briefly, a part of the tissue was digested three times with a mixture of deionized water, HNO_3 and H_2O_2 until almost dryness. The residual mass was finally dissolved in 1% HNO_3 and the solution was used for the estimation of arsenic content by an Atomic Absorption Spectrophotometer (Perkin Elmer Model No. 3100) furnished with an arsenic hollow cathode lamp.

Determination of kidney weight to body weight ratio

Each animal was weighed prior to sacrifice. After sacrifice, the kidneys from all the animals were quickly excised and weighed. The ratio of kidney weight to body weight was then determined for each animal.

Assessment of serum specific markers related to renal dysfunction

For the assessment of serum-specific markers (creatinine and UN levels) related to renal damage, blood samples were collected by puncturing the hearts of all animals (four groups, consisted of six rats in each), kept overnight for clotting and then centrifuged at 3000 g for 10 min. Creatinine and UN levels in the sera were measured by using standard kits.

Preparation of kidney homogenate

Kidneys were homogenized using a glass homogenizer in a buffer (100 mM potassium phosphate, 1 mM EDTA, pH 7.4) and centrifuged at 12 000 g for 30 min at 4°C. The supernatant was collected and used for the experiments.

Determination of protein content

The protein content of the experimental samples was measured by the method of Bradford [17] using crystalline BSA as standard.

Measurement of intracellular ROS production, TNF-α and nitric oxide (NO) levels

Intracellular ROS production was estimated by using 2,7-dichlorofluorescein diacetate (DCFDA) as a probe and following the method as described elsewhere [18,19]. Briefly, 100 μl of kidney homogenates were incubated with the assay media (20 mM tris-HCl, 130 mM KCl, 5 mM $MgCl_2$, 20 mM NaH_2PO_4, 30 mM glucose and 5 μM DCFDA) at 37°C for 15 min; 1 μmol of H_2O_2 was added into the mixture at the end of the assay. The formation of DCF was measured at the excitation wavelength of 488 nm and emission wavelength of 510 nm for 10 min by using a fluorescence spectrometer (HITACHI, Model No F4500).

TNF-α levels were determined from the sera of normal and experimental animals using sandwich ELISA following the method of Ausubel et al. [20].

The serum NO level was indirectly assessed by measuring the nitrite levels in plasma using a colourimetric method based on the Griess reaction [21].

Determination of in vivo *antioxidant power by FRAP assay*

The *in vivo* antioxidant power in the renal tissue of the experimental animals (four groups, consisted of six rats in each, 12 weeks old) was determined following the method of Benzie and Strain [22]. Briefly, 50 µl of sample was added to 1.5 ml freshly prepared and pre-warmed (37°C) FRAP reagent (300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in the ratio of 10:1:1) and incubated at 37°C for 10 min. The absorbance of the sample was recorded against reagent blank (1.5 ml FRAP reagent + 50 µl distilled water) at 593 nm.

Estimation of MDA, lipid hydroperoxide and protein carbonyl content

The lipid peroxidation in terms of malondialdehyde (MDA) was measured according to the method of Esterbauer and Cheeseman [23]. The amount of hydroperoxide produced was calculated using the molar extinction coefficient of 4.6×10^4 $M^{-1}cm^{-1}$.

The concentration of lipid hydroperoxide in the experimental samples was estimated by the FOX assay as described by Jiang et al. [24]. The amount of hydroperoxide produced was calculated using the molar extinction coefficient of 4.6×10^4 $M^{-1}cm^{-1}$.

Protein carbonyl contents were determined according to the methods of Uchida and Stadtman [25]. The results were calculated based on the molar extinction coefficient of 22 000 $M^{-1}cm^{-1}$ for aliphatic hydrazones and expressed as nmol of DNPH incorporated/mg protein.

Assay of antioxidant enzymes and cellular metabolites

The activities of antioxidant enzymes, SOD, CAT, GST, GR, GPx and G6PD as well as the levels of cellular metabolites were measured in kidney homogenates of all experimental animals.

SOD activity was measured by following the method originally developed by Nishikimi et al. [26] and then modified by Kakkar et al. [27]. One unit of SOD activity is defined as the enzyme concentration required to inhibit chromogen production by 50% in 1 min under the assay conditions.

CAT activity was determined by measuring the decomposition of H_2O_2 at 240 nm for 10 min and was monitored spectrophotometrically according to the method of Bonaventura et al. [28]. One unit of CAT activity is defined as the amount of enzyme which reduces 1 µmol of H_2O_2 per minute.

GST activity was assayed based on the conjugation reaction with glutathione in the first step of mercapturic acid synthesis [29]. The GST activity was expressed as µmoles of CDNB conjugate formed/min/mg protein.

GR activity was determined according to the method of Smith et al. [30]. The increase in absorbance at 412 nm was monitored spectrophotometrically for 3 min at 24°C. The enzyme activity was calculated using molar extinction coefficient of 13 600 $M^{-1}cm^{-1}$. One unit of enzyme activity is defined as the amount of enzyme which catalyses the oxidation of 1 µmol NADPH per minute.

GPx activity was measured following the method of Flohe and Gunzler [31] using H_2O_2 and NADPH as substrates. The conversion of NADPH to $NADP^+$ was observed by recording the changes in absorption intensity at 340 nm. One unit of enzyme activity is defined as the amount of enzyme that catalyses the oxidation of 1 µmol NADPH per minute.

G6PD activity was determined following the method as described by Lee [32]; 50 µl of tissue homogenate was mixed with the reaction cocktail (0.1 M Tris-HCl buffer, pH 8.0, containing 1 mM glucose-6-phosphate, 1 mM $NADP^+$) and the reaction mixture was incubated for 5 min at 25°C. The conversion of $NADP^+$ to NADPH was observed by recording the changes in absorption intensity at 340 nm. One unit of G6PD activity was calculated as 1 nmol of $NADP^+$ converted in NADPH per minute.

GSH level was measured according to the method of Ellman [33] by using DTNB (Ellman's reagent) as the key reagent. A standard curve was drawn using different known concentrations of GSH solution. With the help of this standard curve, GSH contents were calculated.

GSSG contents were determined by following the method of Hissin and Hilf [34]. The results were expressed as nmol per mg protein.

Total thiol (sulphhydryl groups) content was measured according to the method of Sedlak and Lindsay [35] with some modifications. The content of total thiols was calculated using molar extinction coefficient of 13 600 $M^{-1}cm^{-1}$.

DNA fragmentation assay

The extent of DNA fragmentation (DNA ladder formation) was determined by electrophoresing genomic DNA samples, isolated from the kidneys of normal and experimental rats, on agarose/EtBr gel by the procedure as described by Sellins and Cohen [36].

Immunoblotting

For immunoblotting, sample were prepared by homogenizing the frozen renal tissues in lysis buffer (50 mmol/L Tris, 15 mmol/L EDTA, 150 mmol/L NaCl, 0.1%Triton X-100, pH 8.0) containing protease inhibitors (2 µg/mL Leupeptin and 1 mmol/L phenylmethylsulphonyl fluoride, PMSF) at a ratio of 1:15 w/v and incubating the mixture on ice for 30 min. After centrifugation at 12 000 g (4°C) for 20

min, the supernatant was carefully recovered and stored at -80°C prior to electrophoresis.

Proteins (50 μg) from the supernatant of the experimental samples were loaded on 10% SDS-PAGE and transferred to nitrocellulose membranes. Membranes were blocked at room temperature for 2 h in blocking buffer containing 5% non-fat dry milk and then incubated with anti p-38 (1:1000 dilution), anti ERK1/2 (1:1000 dilution), anti-NF-κB (p65) (1:250 dilution), anti BAD (1:1000 dilution) and anti Bcl-2 (1:1000 dilution) primary antibodies at 4°C overnight as needed. The membranes were washed in TBST (50 mmol/L Tris-HCl, pH 7.6, 150 mmol/L NaCl, 0.1% Tween 20) for 30 min and incubated with appropriate HRP conjugated secondary antibody (1:2000 dilution) for 2 h at room temperature and developed by the HRP substrate 3,3'-diaminobenzidine tetrahydrochloride (DAB) system (Bangalore genei, India).

Determination of mitochondrial membrane potential ($\triangle\Psi_m$)

Fresh mitochondria were isolated from the kidney tissues following the method of Hodarnau et al. [37]. Mitochondrial membrane potentials ($\triangle\Psi_m$) of the experimental samples were then estimated on the basis of cell retention of the fluorescent cationic probe rhodamine 123 [38]. Briefly, the mitochondrial suspension was incubated with 1 μM rhodamine 123 for 10 min, centrifuged at 50 *g* for 5 min at 4°C, washed and resuspended in 1 ml of 0.1% Triton X-100. After centrifugation at 2000 *g* for 5 min, fluorescence of rhodamine 123 was determined in the supernatant with a fluorescence spectrophotometer (Hitachi, Tokyo, Japan) at the 505/535-nm excitation/emission wavelength pair.

Assay of cytochrome C release

Mitochondrial cytochrome C was measured following the method described by Adrain et al. [39] using a cytochrome C enzyme immunometric assay kit.

Histological studies

Kidneys from the normal and experimental rat were fixed in 10% buffered formalin and processed for paraffin sectioning. Sections of ~5 μm thickness were stained with haematoxylin and eosin to evaluate ultrastructural changes of the renal tissues under a light microscope.

Statistical analysis

All the values are represented as mean ± SD ($n = 6$). Data on biochemical investigation were analysed using analysis of variance (ANOVA) and the group means were compared by Duncan's Multiple Range Test (DMRT). *P*-values of 0.05 or less were considered significant.

Results

As-induced renal pathophysiology: Prevention by taurine

Figure 1 showed that administration of $NaAsO_2$ decreased SOD activity linearly up to a dose of 3 mg/kg body weight in 2 weeks. On the other hand, Figure 2 showed that taurine administration increased intracellular antioxidant power linearly up to a dose of 100 mg/kg body weight, when applied for 5 days prior to $NaAsO_2$ intoxication. These doses and times were, therefore, chosen as the optimum dose and time of $NaAsO_2$ and taurine throughout the study.

Increased glomerular area, glomerular volume, blood urea nitrogen (BUN), serum creatinine and reduction of the organ-to-body weight ratio as well as the accumulation of the toxic metals are the parameters representing renal dysfunction. These para-

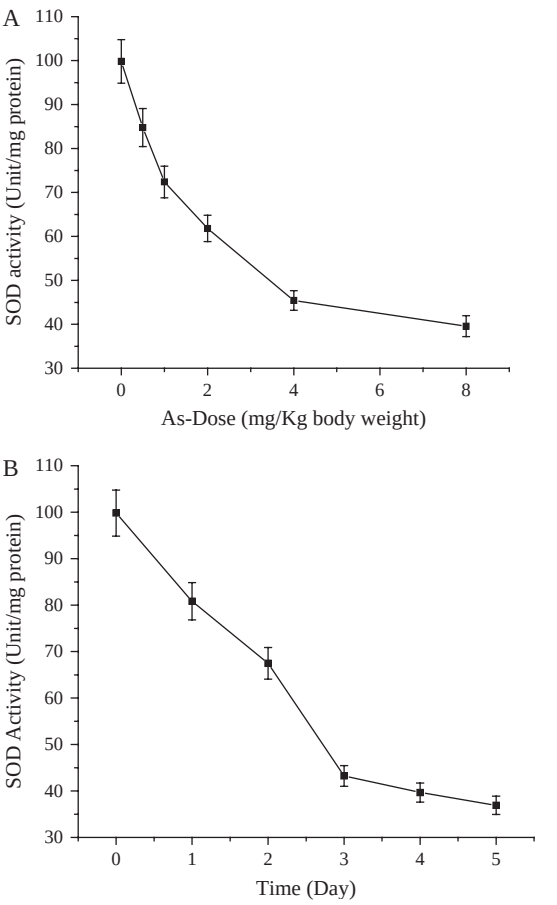

Figure 1. (A) Dose-dependent effect of arsenic on SOD activity. SOD activity was determined in the renal tissue of arsenic intoxicated rats for 3 days at a dose of 0.5, 1, 2, 4 and 8 mg/kg body weight. (B) Time-dependent effect of arsenic on SOD activity. SOD activity was determined in the renal tissue in arsenic intoxicated rat (4 mg/kg body weight, orally) for 1, 2, 3, 4 and 5 days, respectively. Each point represents mean ± SD, $n = 6$. pa < 0.01 significantly different from the group marked '0'.

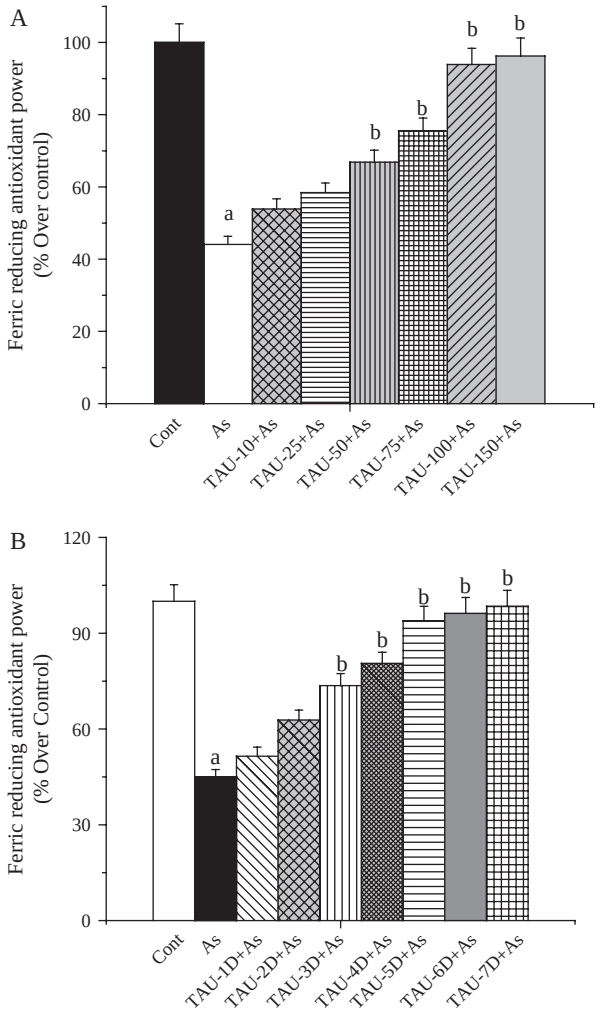

Figure 2. (A) Dose-dependent effect of taurine on intracellular antioxidant power against As-induced toxicity in the renal tissue of the experimental rat. Cont: antioxidant power in normal rat, $NaAsO_2$: antioxidant power in cadmium treated rat, TAU-10+As, TAU-25+As, TAU-50+As, TAU-75+As, TAU-100+As, TAU-150+As: antioxidant power in taurine (TAU) treated rat for 5 days at a dose of 10, 25, 50, 75, 100 and 150 mg/kg body weight prior to $NaAsO_2$ intoxication. (B) Time-dependent effect of taurine on intracellular antioxidant power against As induced toxicity in the renal tissue of the experimental rat. Cont: antioxidant power in normal rat, $NaAsO_2$: antioxidant power in $NaAsO_2$ treated rat, TAU-1D+ $NaAsO_2$, TAU-2D+ $NaAsO_2$, TAU-3D+ $NaAsO_2$, TAU-4D+ $NaAsO_2$, TAU-5D+ $NaAsO_2$, TAU-6D+ $NaAsO_2$ and TAU-7D+ $NaAsO_2$: antioxidant power in taurine (TAU) treated rat for 1, 2, 3, 4, 5, 6 and 7 days, respectively, at a dose of 100 mg/kg body weight prior to $NaAsO_2$ intoxication. Each column represents mean $\pm$ SD, $n = 6$. 'a' indicates the significant difference between the normal control and toxin-treated groups and 'b' indicates the significant difference between the toxin-treated and taurine pre-treated groups. ($p^a < 0.01$, $p^b < 0.01$).

meters were, therefore, measured in the experimental animals. Results suggest that toxin exposure caused significant accumulation of metallic As in the renal tissue and lowered the kidney weight-to-body weight ratio. The same exposure also caused renal dysfunction by enhancing glomerular area, glomerular volume, BUN and creatinine. Taurine treatment could, however, prevent the alterations of these changes, as shown in Table I.

As-induced renal oxidative stress: Prevention by taurine

In order to assess whether taurine could imply any effect on As-induced ROS production, level of intracellular ROS in different experimental kidney tissues has been estimated and presented in Table II. The rate of ROS production in the $NaAsO_2$ treated group was found to be significantly higher than that of the normal animals. Similar results were also observed on NO production and TNF-α release. Taurine treatment, however, prevented these alterations.

Lipid peroxidation and protein carbonyl contents are two important widely used indicators of free radical-induced oxidative stress-related cell membrane damage and oxidative modification of proteins. Table III represents the changes in lipid peroxidation and protein carbonylation in the renal tissue of the experimental animals. $NaAsO_2$ exposure increased the levels of MDA, lipid hydroperoxide and protein carbonylation. Pre-treatment with taurine was found to be effective in preventing $NaAsO_2$ induced lipid peroxidation and protein carbonylation.

Intracellular antioxidant enzymes and metabolites are considered to be the first and second line of cellular defence, respectively, in oxidative stress-induced organ pathophysiology. The activities of antioxidant enzymes, namely SOD, CAT, GST, GR, GPx and G6PD, as well as the levels of GSH, GSSG and total thiols were, therefore, determined in the renal tissues of the experimental animals. The results have been summarized in Tables IV and V. Significant reduction in the activities of all these enzymes and levels of GSH and total thiols were observed in the renal tissue of the experimental animals exposed to $NaAsO_2$; however, taurine treatment prevented these alterations.

As-induced cell death: Protective role of taurine

In order to investigate the nature of As-induced cell death, we began with DNA fragmentation analysis. Results showed that arsenic intoxication caused DNA ladder formation, a definite index of apoptosis (Figure 3). This observation helped us further in investigating arsenic-induced oxidative stress responsive cell death pathways. Intracellular oxidative-stress stimuli can activate both MAPKs and NF-κB. So, we checked whether any member of the MAPK family is involved in As-induced nephrotoxicity and its prevention by taurine. Immunoblot analysis showed (Figure 4A and B) that arsenic administration stimulated the expression of phospho-p38, phospho-ERK1/2. However, significant changes in the expression of total p38 and ERK 1/2 were not observed between the normal and arsenic treated groups. In addition, we observed that arsenic intoxication

Table I. Effects of arsenic (As) and taurine (Tau) on body weight (BW), kidney weight (KW), KW/BW, glomerular area, glomerular volume, blood urea nitrogen (BUN) and creatinine.

Parameters	Normal control	As treated	Tau treated	Tau+As
Body weight (BW) (g)	148.33±7.56	108.25±5.42[a]	145.12±7.23	136.11±6.79[b]
Kidney weight (KW) (g)	1.22±0.061	1.57±0.078[a]	1.26±0.062	1.28±0.064[b]
KW/BW (%)	0.81±0.041	1.45±0.072[a]	0.86±0.043	0.94±0.047[b]
Glomerular area (μm^2, $\times 10^3$)	4.62±0.25	6.72±0.34[a]	4.61±0.29	5.32±0.31[b]
Glomerular volume (μm^2, $\times 10^3$)	2.39±0.18	3.43±0.21[a]	2.46±0.18	2.64±0.14[b]
BUN (mg/dL)	22.31±1.05	215.85±15.31[a]	20.44±1.03	116.26±5.91[b]
Creatinine (mg/dL)	0.61±0.029	2.21±0.09[a]	0.61±0.031	0.88±0.045[b]

Values are expressed as mean±SD, for six animals in each groups.
[a] The significant difference between the normal control and arsenic-treated groups; [b] The significant difference between the arsenic-treated and taurine pre-treated groups; $p^a < 0.05$, $p^b < 0.05$.

Table II. Effect of Arsenic (As) and taurine (Tau) on the rate of ROS production, the levels of serum TNF-α and NO production.

Parameters	Normal control	As treated	Tau	Tau+As
Rate of intracellular ROS production (nmols of DCF/min/mg protein)	31.12±1.45	140.48±6.95[a]	32.38±1.67	54.25±2.65[b]
TNF-α (pg/ml serum)	9.48±0.51	49.26±2.39[a]	12.12±0.59	25.32±1.24[b]
NO production (mM)	52.28±2.71	181.85±8.88[a]	61.37±3.01	82.85±4.51[b]

Values are expressed as mean±SD, for six animals in each groups.
[a]The significant difference between the normal control and arsenic-treated groups; [b] The significant difference between the arsenic-treated and taurine pre-treated groups; $p^a < 0.05$, $p^b < 0.05$.

Table III. Effect of arsenic (As) and taurine (Tau) on the levels of MDA, lipid peroxide and protein carbonylation in the kidney tissues of the normal and experimental animals.

Parameters	Normal control	As treated	Tau treated	Tau+As
MDA (nmol/mg protein)	4.41±0.27	13.76±0.71[a]	4.79±0.25	6.25±0.37[b]
Lipid hydroperoxide (nmol/mg protein)	6.66±0.35	18.47±0.98[a]	6.94±0.37	9.47±0.55[b]
Protein carbonylation (nmol/mg protein)	4.35±0.24	12.35±0.72[a]	4.45±0.28	6.25±0.35[b]

Values are expressed as mean±SD, for six animals in each groups.
[a]The significant difference between the normal control and arsenic-treated groups; [b] The significant difference between the arsenic-treated and taurine pre-treated groups; $p^a < 0.05$, $p^b < 0.05$.

Table IV. Effect of arsenic (As) and taurine (Tau) on the activities of the antioxidant enzymes in the kidney tissues of the normal and experimental animals.

Name of the antioxidant enzymes	Normal control	As treated	Tau treated	Tau+As
SOD (Unit/mg protein)	4.61±0.24	0.83±0.09[a]	3.97±0.22	3.57±0.19[b]
CAT (μmol/min/mg protein)	101.36±5.29	51.48±2.32[a]	105.69±9.51	96.37±4.75[b]
GST (μmol/min/mg protein)	0.42±0.022	0.19±0.009[a]	0.39±0.018	0.35±0.015[b]
GR (nmol/min/mg protein)	31.25±1.51	11.34±0.65[a]	30.97±1.49	28.34±1.45[b]
GPx (nmol/min/mg protein)	72.21±3.52	22.19±1.11[a]	65.21±3.27	59.34±2.79[b]
G6PD (nmol/min/mg protein)	4.11±0.25	1.79±0.11[a]	3.78±0.21	3.67±0.18[b]

Values are expressed as mean±SD, for six animals in each group.
[a]The significant difference between the normal control and arsenic-treated groups; [b] The significant difference between the arsenic-treated and taurine pre-treated groups; $p^a < 0.05$, $p^b < 0.05$.

Table V. Status of the thiol-based antioxidant in the renal tissue of the arsenic (As) and taurine (Tau) treated animals.

Parameters	Normal control	As treated	Tau treated	Tau+As
GSH (μmol/mg protein)	8.14±0.42	2.92±0.18[a]	7.52±0.39	6.55±0.34[b]
GSSG (μmol/mg protein)	0.29±0.015	0.85±0.047[a]	0.28±0.013	0.49±0.022[b]
Total thiols (μmol/mg protein)	127.65±6.38	59.91±3.02[a]	111.71±5.89	102.39±5.22[b]

Values are expressed as mean±SD, for six animals in each group.
[a]The significant difference between the normal control and arsenic-treated groups; *b* The significant difference between the arsenic-treated and taurine pre-treated groups; $p^a < 0.05$, $p^b < 0.05$.

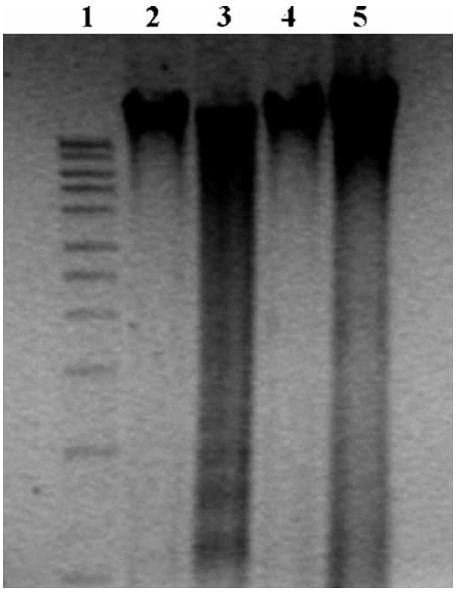

Figure 3. DNA fragmentation analysis on agarose/ethydium bromide gel. DNA isolated from experimental kidney tissues was loaded onto 1% (w/v) agarose gels. Lane 1: Marker (1 kb DNA ladder); Lane 2: DNA isolated from normal kidney; Lane 3: DNA isolated from the kidney tissue of animals treated with As; Lane 4: DNA isolated from the kidney tissue of the taurine animals; and Lane 5: DNA isolated from the kidney tissue of the animals treated with taurine prior to arsenic intoxication.

induced the expressions of NF-κB (p65) (Figure 5). Treatment with taurine down-regulated the activation of phospho-p38, phospho-ERK1/2 and NF-κB induced by arsenic.

It is known that oxidative stress can disrupt mitochondrial membrane potential ($\triangle\Psi_m$) and enhance the release of cytochrome C and ultimately lead the cells to apoptotic death. Besides, apoptosis is considered to be regulated by a complex interplay of pro-apoptotic and anti-apoptotic mitochondrial membrane proteins (Bcl-2 family proteins). To determine whether taurine exerts its anti-apoptotic action against As-induced cellular death via this mechanism, we investigated the status of these targets in As-intoxicated renal tissues. Results showed that As significantly attenuated the mitochondrial membrane potential and elevated the concentration of cytosolic cytochrome C (Figure 6A and B). Immunoblotting studies also demonstrated that As upregulated the pro-apoptotic (Bad) and down-regulated the anti-apoptotic (Bcl-2) Bcl-2 family proteins in the renal tissue (Figure 7). Treatment with taurine prior to the $NaAsO_2$ administration could, however, inhibit As-induced alterations of these signalling molecules.

Histological assessment

Histological studies showed that $NaAsO_2$ exposure caused multiple foci of haemorrhage, necrosis and cloudy swelling of tubules in the kidney tissue (Figure 8B). Treatment with taurine reduced the $NaAsO_2$ induced pathological lesions (Figure 8C) and that is in agreement with the results of the other parameters.

Discussion

Increasing evidence suggest that the kidney is extremely vulnerable to oxidative stress due to the high concentration of non-heme iron that is catalytically involved in the production of free radicals [40]. High rate of oxygen utilization and abundant supply of poly unsaturated fatty acids (PUFAs) make this particular organ susceptible towards oxidative stress [41]. In the present study we observed that $NaAsO_2$ decreased kidney weight-to-body weight ratio and increased the intracellular concentration of metallic arsenic in the kidney tissue of the toxin treated rats. In addition, As induced oxidative imbalance and apoptotic cell death by altering various pro-oxidant–antioxidant parameters. Antioxidant defence machineries in our body operate for scavenging ROS to prevent the oxidative stress. Among the different antioxidant molecules, SOD and CAT mutually function as important enzymes in the elimination of ROS. On the other hand, GSH maintains the intracellular redox status against pro-oxidative stress by detoxifying various xenobiotics as well as by scavenging free radicals. GST and GPx also utilize GSH during their course of reactions. Arsenic exposure inactivates most of the antioxidant molecules either by the direct binding of the metal to the active sites containing –SH groups [42] or by the displacement of the metal cofactors from the active sites [43]. Decrease in GSH content due to arsenic toxicity simultaneously decreased the activities of GST as well as GPx, with a concomitant decrease in the activity of GSH regenerating enzyme, GR. As-induced increased lipid peroxidation plays an important role in the oxidative stress-related organ pathophysiology. In line with the earlier findings, we also observed that activities of the antioxidant enzymes in the kidney tissue of the experimental rats have been significantly decreased in association with enhanced lipid peroxidation and protein carbonylation due to arsenic toxicity. The results suggest the inability of the host's antioxidant defense machineries to counteract the oxidative stress induced by As exposure. Besides, a significant decrease in the levels of GSH as well as total thiols and simultaneous increase in GSSG level have been observed in the kidney tissue of the $NaAsO_2$-exposed animals. It is probably due to the increased utilization of thiol containing substances to overwhelm the free radicals and lipid peroxidation products. Taurine supplementation could, however, prevent the As-induced alterations of these antioxidant enzymes activities, lipid peroxidation and GSH levels. Protection of glutathione redox ratio in the kidney tissue suggests a novel protective action of taurine in

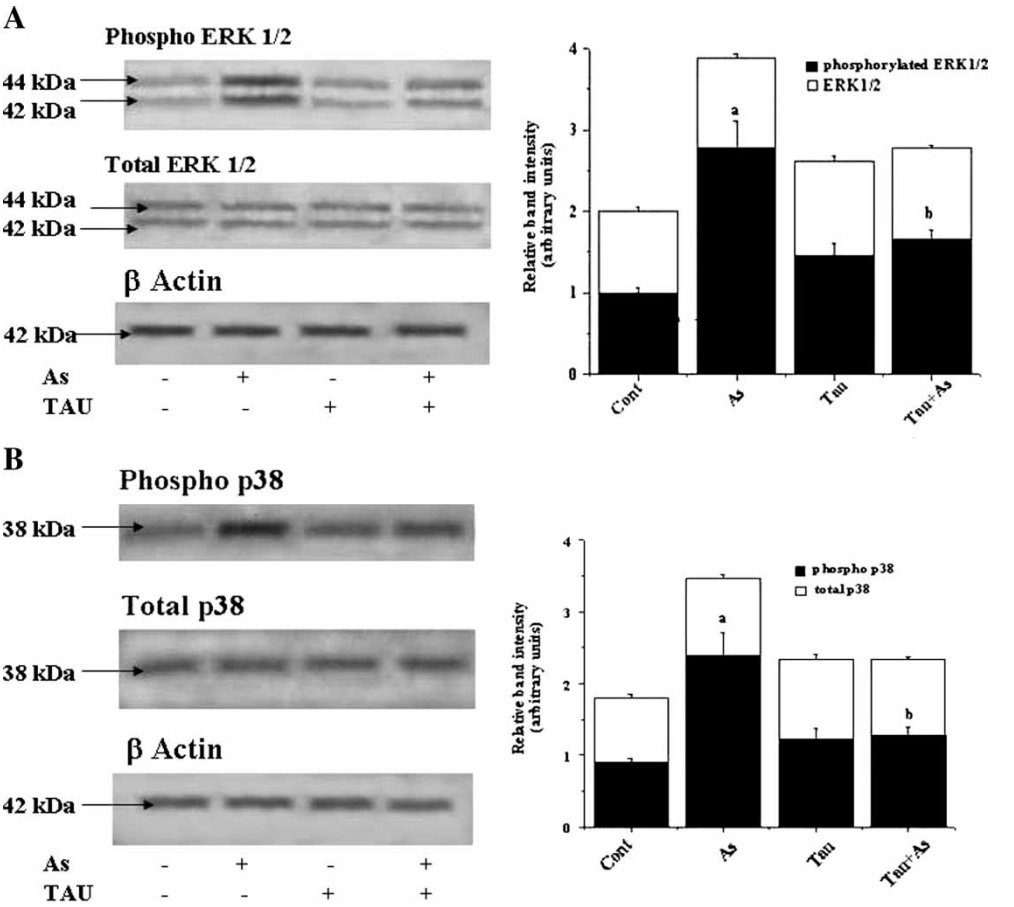

Figure 4. Effect of taurine on As-induced phosphorylation of (A) ERK ½ and (B) p38. Left panel represents the western blot and right panel represents the relative band intensity. The relative intensities of bands were determined using NIH-image software and the control band was given an arbitrary value of 1. The results shown in the right panels are the average ± SD, $n = 6$. Values with no common superscript are significant. 'a' indicates the significant difference between the normal control and As-treated groups and 'b' indicates the significant difference between the As-treated and taurine pre-treated groups ($p^a < 0.05$, $p^b < 0.05$).

increased renal oxidative stress. Further evidence in As-induced renal oxidative stress and the protective role of taurine (as an antioxidant in this pathophysiology) came from the results of FRAP assay. In the present study we observed that As intoxication increased the rate of DCF formation, an indicator

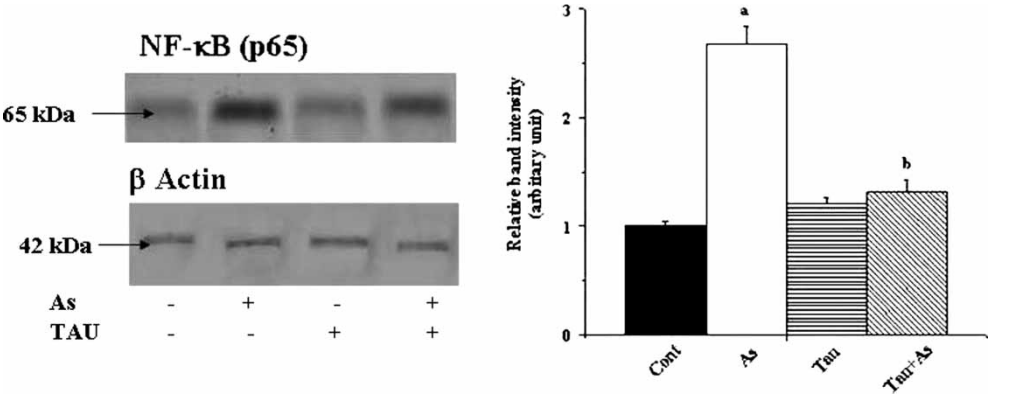

Figure 5. Effect of taurine on As-induced induced activation of NF-κB (p65). Left panel represents the western blot and right panel represents the relative band intensity. The relative intensities of bands were determined using NIH-image software and the control band was given an arbitrary value of 1. The results shown in the right panel are the average ± SD, $n = 6$. Values with no common superscript are significant. 'a' indicates the significant difference between the normal control and As-treated groups and 'b' indicates the significant difference between the As-treated and taurine pre-treated groups ($p^a < 0.05$, $p^b < 0.05$).

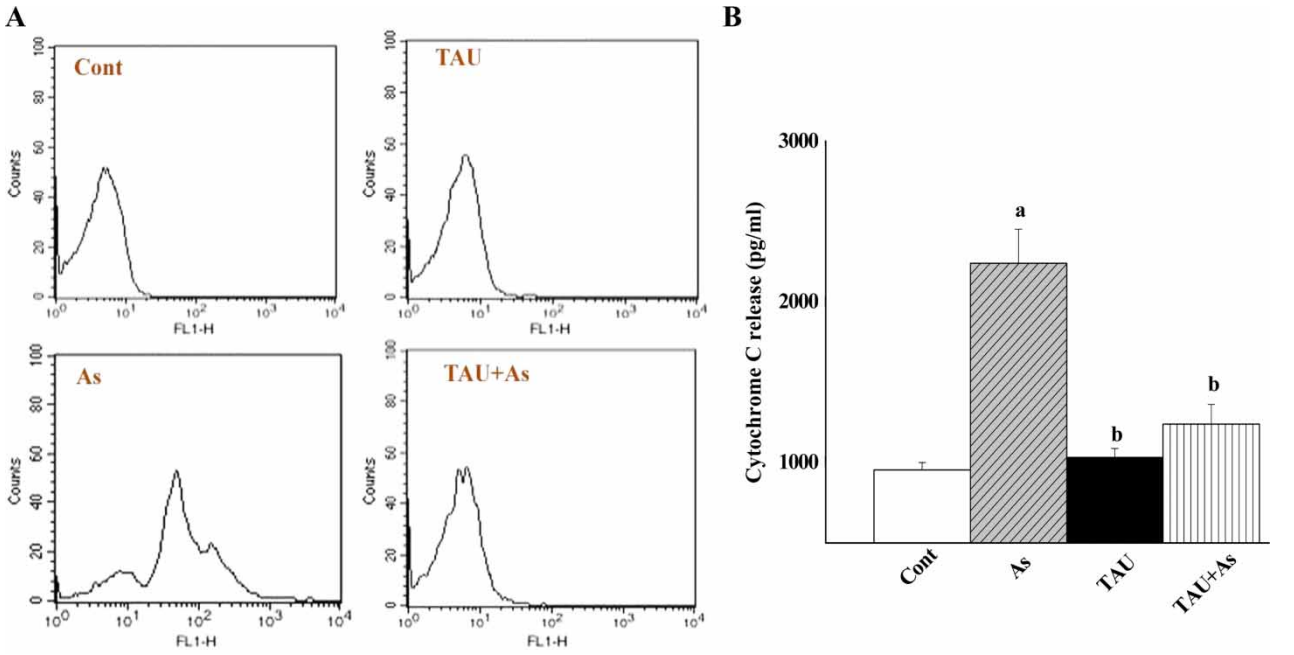

Figure 6. (A) Study on the mitochondrial membrane potential ($\triangle\Psi_m$) by flow cytometry analysis. Figures represent histogram plots. Results expressed as a dot plot representing one of the six independent experiments. Cont: $\triangle\Psi_m$ value in the normal rats, As: $\triangle\Psi_m$ value in As-treated rats, TAU: $\triangle\Psi_m$ value in rats treated with taurine, As+TAU: $\triangle\Psi_m$ value in rats treated with taurine prior to As administration. (B) Effect of taurine on As-induced release of cytochrome C. Cont: cytochrome C level in normal rats, As: cytochrome C level in As treated rats, TAU: cytochrome C level in rats treated with TAU, TAU+As: cytochrome C level in rats treated with TAU prior to As administration. Each column represents mean±SD, $n=6$. 'a' indicates the significant difference between the normal control and As-treated groups, 'b' indicates the significant difference between the As-treated and TAU-treated as well as TAU pre-treated groups (TAU+As) ($p^a<0.05$, $p^b<0.05$).

of intracellular ROS production. Pre-treatment with taurine could, however, prevent the increased production of ROS and maintain the intracellular antioxidant power close to normal.

Another harmful effect of ROS can be inducing DNA damage in organ pathophysiology. ROS-induced DNA damage can produce a multiplicity of modifications (like base and sugar lesions, strand breaks, DNA-protein cross-links and base-free sites) in DNA and, if left unrepaired, this can lead to detrimental biological consequences in organisms including mutations and transformation of cells to malignant cells and cell death. DNA ladder formation by agarose gel electrophoresis is a dependable method for the detection of the nature (apoptotic or necrotic) of cell death in oxidative stress-related organ

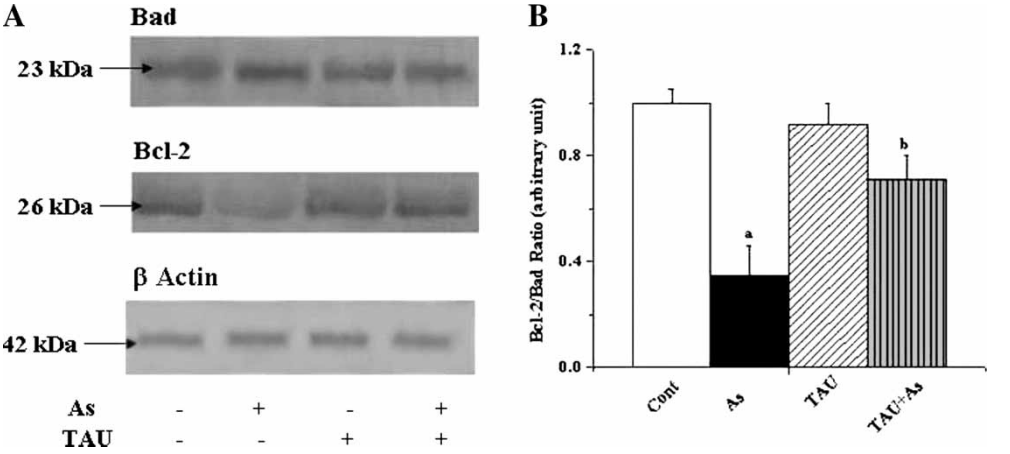

Figure 7. Western blot analyses of Bcl-2 family proteins in response to As and taurine in kidney tissue homogenates. (A) Bad and Bcl-2, (B) ratio of Bad and Bcl-2. Data represent the average±SD of six separate experiments in each group. 'a' indicates the significant difference between the normal control and As-treated groups, 'b' indicates the significant difference between the As-treated and taurine pre-treated groups (TAU+ As) ($p^a<0.05$, $p^b<0.05$).

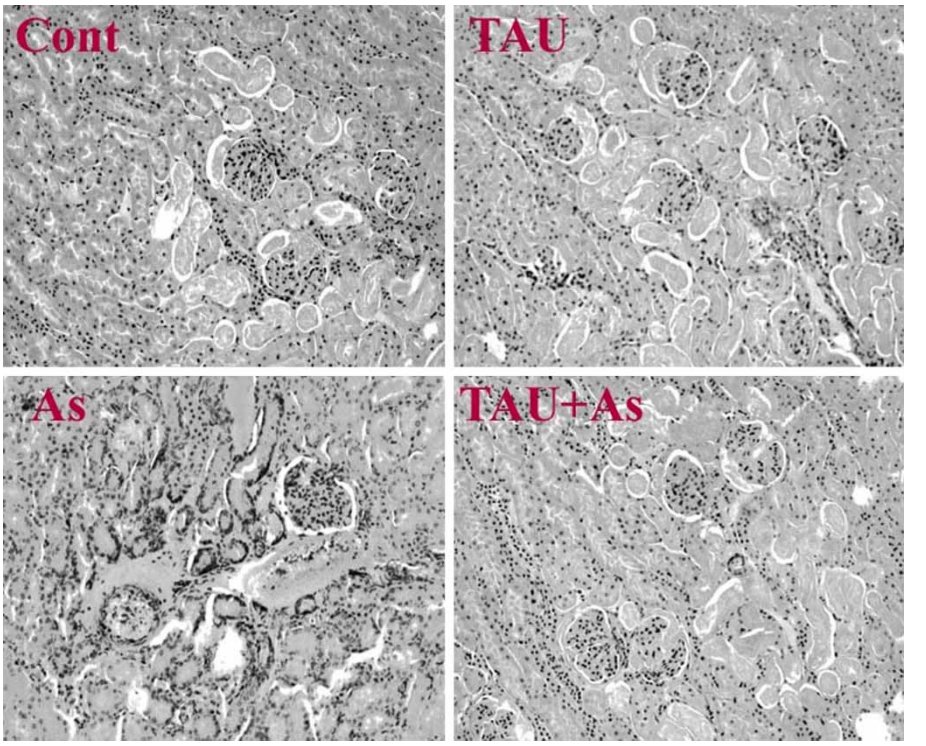

Figure 8. Haematoxylin and eosin stained kidney sections. (Cont) normal rat kidney (×100), (As) Toxin ($NaAsO_2$) control (×100), (TAU) taurine-treated kidney section (×100), (TAU+As) kidney section of taurine pre-treated animals (×100).

pathophysiology. While the appearance of DNA ladder fragmentations in agarose gel indicates apoptotosis, the appearance of a smear in the same indicates necrosis. In order to find out whether taurine protects As-induced apoptotic or necrotic cell death, DNA isolated from the renal tissues of experimental rats were subjected to agarose gel electrophoresis. As-intoxicated animals caused a DNA ladder fragmentation, a hallmark of apoptosis. However, in the taurine treated animals, this change in DNA was practically absent, suggesting that As-induced apoptotic DNA damage could be prevented by this amino acid. To further analyse the molecular mechanism underlying the protective effect of taurine in As-induced oxidative renal dysfunction and cell death, we began investigating the role of MAPK family proteins in this pathophysiological situation and observed that As intoxication upregulated the expression of phospho-ERK1/2 and phospho-p38, keeping the expression of total ERK1/2 and p38 almost unchanged. Earlier studies also suggest that NF-κB dependent NO production is a critical component in the oxidative stress-related organ pathophysiology. Since arsenic induces NO production in our experiments, we planned to investigate whether NF-κB activation is indeed involved in As-induced free radical-mediated renal pathophysiology. Results suggest that As induced the upregulation of NF-κB (p65) in the renal tissue of As intoxicated animals. Treatment with taurine could, however, inhibit or reduce that upregulation of phospho-ERK 1/2, phospho-p38 and NF-κB (p65). The activation of phospho-ERK1/2 and phospho-p38 were suggested to be a critical component in the oxidative stress-induced apoptotic process. Oxidative stress can increase the permeability of the mitochondrial membrane, resulting in the disruption of the mitochondrial membrane potential and enhancement of the release of cytochrome C, which in turn could cause the cleavage of a variety of important molecules, like PARP (an endogenous caspase substrate) and ultimately causes apoptotic cell death. Bcl-2 family proteins are upstream regulators of mitochondrial membrane potential. There are two classes of regulatory proteins in the Bcl2 family that confer opposite effects on apoptosis: the anti-apoptotic members (e.g. Bcl2, BclxL) protect cells against apoptosis, whereas the pro-apoptotic members (e.g. Bax, Bad) promote programmed cell death. The pro-apoptotic members of Bcl-2 family proteins physically interact to form oligomers that can move onto the mitochondrial membrane and release cytochrome C. To determine whether taurine exerts its anti-apoptotic action via this mechanism, we determined the expressions of Bcl-2 family proteins, mitochondrial membrane potential and cytosolic cytochrome C. Results suggest that arsenic intoxication up-regulated pro-apoptotic (Bad) and down-regulated anti-apoptotic (Bcl-2) proteins. In addition, it also caused pronounced disruption of the mitochondrial membrane potential

and enhanced the release of cytochrome C. However, taurine treatment effectively suppressed these alterations of mitochondrial events, thereby suggesting its role as an anti-apoptotic agent in As-induced renal pathophysiology.

Histological examination revealed that arsenic intoxication caused significant damage in renal ultra structure, showing marked tubular damages. Complete loss of brush borders, extensive tubular casts and debris as well as tubular dilatations were observed in the renal tissue of As-intoxicated animals. Treatment with taurine prevented any such alterations and kept the kidney histologically sound.

In conclusion, the results of our study strongly suggest that As not only increased phosphorylation of p38 and ERK ½, but also led to NF-κB translocation in oxidative stress-mediated renal dysfunctions. This interaction could be a cross-talk site among the NF-κB, MAPKs and other related signalling pathways. Taurine treatment mitigated the activation of these pathways and blocked the apoptotic signalling cascades. In other words, taurine supplementation represents a promising approach for the protection of the organ tissue in As-induced renal injury and cell death.

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