Taurine exerts hypoglycemic effect in alloxan-induced diabetic rats, improves insulin-mediated glucose transport signaling pathway in heart and ameliorates cardiac oxidative stress and apoptosis

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ABSTRACT

Hyperlipidemia, inflammation and altered antioxidant profiles are the usual complications in diabetes mellitus. In the present study, we investigated the therapeutic potential of taurine in diabetes associated cardiac complications using a rat model. Rats were made diabetic by alloxan (ALX) (single i.p. dose of 120 mg/kg body weight) and left untreated or treated with taurine (1% w/v, orally, in water) for three weeks either from the day of ALX exposure or after the onset of diabetes. Animals were euthanized after three weeks. ALX-induced diabetes decreased body weight, increased glucose level, decreased insulin content, enhanced the levels of cardiac damage markers and altered lipid profile in the plasma. Moreover, it increased oxidative stress (decreased antioxidant enzyme activities and GSH/GSSG ratio, increased xanthine oxidase enzyme activity, lipid peroxidation, protein carbonylation and ROS generation) and enhanced the proinflammatory cytokines levels, activity of myeloperoxidase and nuclear translocation of NF-κB in the heart tissue of the experimental animals. Taurine treatment could, however, result to a decrease in the elevated blood glucose and proinflammatory cytokine levels, diabetes-evoked oxidative stress, lipid profiles and NF-κB translocation. In addition, taurine increased GLUT 4 translocation to the cardiac membrane by enhanced phosphorylation of IR and IRS1 at tyrosine and Akt at serine residue in the heart. Results also suggest that taurine could protect cardiac tissue from ALX induced apoptosis via the regulation of Bcl2 family and caspase 9/3 proteins. Taken together, taurine supplementation in regular diet could play a beneficial role in regulating diabetes and its associated complications in the heart.

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Introduction

Diabetes is the most common and serious metabolic disease. Diabetes is mainly found to be of two types. In type 1, which is also known as IDDM or insulin dependent diabetes mellitus, insulin is produced in lesser amount. In type 2, also known as NIDDM or noninsulin dependent diabetes mellitus, the pancreas is usually producing enough insulin, but for unknown reasons the body cannot use the insulin effectively, a condition called insulin resistance. The chronic disorders related to diabetes apart from hyperglycemia and hyperlipidemia are cardiovascular complications, nephropathy and retina damage (Baynes and Thorpe, 1999; Ceriello, 2000; Yim et al., 2007).

The major role of insulin in diabetes is to maintain whole body glucose homeostasis via glucose transporter 4 (GLUT4), expressed in adipose tissue, skeletal and cardiac muscles (Charron et al., 1999; Haruta et al., 1995). During insulin stimulation, intracellular vesicles that store GLUT4, translocate to the plasma membrane and facilitate glucose uptake (Bryant et al., 2002; Pessin et al., 1999). Under diabetic condition, reduced expression of GLUT4 causes impairment of insulin signaling and stimulates glucose production in the liver. These alterations lead to high glucose concentrations in blood (Nizamutdinova et al., 2009).

Hyperglycemia-induced generation of free radicals (oxidative stress) contributes to the development and progression of diabetes and other related complications. Therefore, an agent that possesses both hypoglycemic and antioxidant activities would be a therapeutic tool for diabetic patients (Manna et al., 2010a,b). Taurine, a sulfur containing beta amino acid, is present in most animal tissues and is available online 26 November 2011

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Abbreviations: ALX, alloxan; CAT, catalase; DAB, 3,3'-diaminobenzidine tetrahydrochloride; EtBr, ethidium bromide; FACS, fluorescence activated cell sorting; GLUT4, glucose transporter type 4; GSH, glutathione; GSSG, glutathione disulfide; GST, glutathione S-transferase; GR, glutathione reductase; HDL, high-density lipoprotein; IL-6, inter leukin 6; IR, insulin receptor; IRS1, insulin receptor substrate 1; LDH, lactate dehydrogenase; LDL, low-density lipoprotein; MDA, malondiadehyde; NF-κB, nuclear factor kappa B; ROS, reactive oxygen species; SOD, superoxide dismutase; STE buffer, sodium chloride-tris-EDTA buffer; TAU, taurine; TNF-α, tumor necrosis factor alpha; TUNEL, terminal transferase mediated dUTP nick end-labeling.

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essential for the normal functioning of different organs (Broznan and Brosnan, 2006). Taurine exhibits antioxidative properties, membrane stabilizing effect, regulates intracellular Ca²⁺ concentration, inhibits apoptosis, reduces the levels of pro-inflammatory cytokines in various organs and controls blood pressure (Aerts and Van Asche, 2002; Das et al., 2008, 2009a,b, 2010a–c, 2011a,b; Kontny et al., 2000; Manna et al., 2008a,b, 2009; Racasan et al., 2004; Sinha et al., 2007, 2008a,b). Taurine is found at high concentrations inside glucagon and somatostatin-containing cells in the pancreatic islets and increases insulin secretion, sensitivity and glucose uptake (Cherif et al., 1998; De la Puerta et al., 2010; Kaplan et al., 2004) in different experimental conditions. Literature suggests a considerable variation about the opinion on the mode of action of taurine in diabetes. Kulakowski and Maturo (1984) reported that the hypoglycemic effect of taurine was not mediated via increased insulin release; on the other hand, Pandya et al. (2010) and Chang and Kwon (2000) reported that taurine increased insulin secretion in streptozotocin-induced diabetic animals. Besides, Brons et al. (2004) reported that taurine supplementation had no effect on insulin secretion and glucose level in prediabetic human. The authors, however, also expressed the opinion that they could not rule out the possibility of hypoglycemic effect of taurine if diabetic subjects had been taken (as reported by Elizarova and Nedosugova (1996)).

The beneficial effects of taurine in diabetes mellitus and its cardioprotective role in other pathophysiological conditions have been known (Das et al., 2011a; Gavrovskaya et al., 2008; Ghosh et al., 2009; Winiarska et al., 2009), although the exact mechanism of hypoglycemic action of taurine in cardiac associated complications is not properly defined. The aim of this present study has, therefore, been set to investigate the mechanisms of possible beneficial action of taurine against ALX (2,4,5,6-tetraoxypyrimidine; 2,4,5,6-pyrimidinetetrone)-induced diabetes and its associated complications in cardiac tissue of rats. In the present study, attempts have, therefore, been made to investigate the mechanisms of hypoglycemic, antioxidant, antiinflammatory and antiapoptotic action of taurine in ALX-induced diabetic heart. The outcome, we believe, might provide important information about its usefulness as a therapeutic agent for the treatment of diabetes and related cardiac dysfunctions.

Materials and methods

Chemicals

Taurine (2-aminoethane sulfonic acid), alloxan, bovine serum albumin (BSA), Bradford reagent, anti Bcl 2, anti Bcl XL, anti caspase 3, anti Akt, and anti NFkB antibodies were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Other antibodies, like, anti IR, anti IRS1, and anti GLUT4 were purchased from Abcam (Cambridge, Cambridgeshire, UK). Kits for measurement of blood glucose, LDH, uric acid and total cholesterol were purchased from Span Diagnostic Ltd., Surat, Gujarat, India. All other chemicals were bought from Sisco Research Laboratory, Andheri, Mumbai, India. TUNEL assay kit was purchased from Invitrogen, Eugene, Oregon, USA.

Animals

Adult male Wistar rats weighing approximately 160–180 g were purchased from M/S Gosh Enterprises, Kolkata, India. Animals were acclimatized under laboratory conditions for two weeks prior to experiments. All the experiments with animals were carried out according to the guidelines of the institutional animal ethical committee (IAEC), Bose Institute, Kolkata and full details of the study were approved by both IAEC and CPCSEA (committee for the purpose of control and supervision on experiments on animals), Ministry of Environment and Forests, New Delhi, India.

Experimental design for in vivo treatments

Experimental design needed for the present in vivo study has been summarized (in Fig. 1) as follows: Forty-five rats were randomly assigned to five groups.

Group 1—Normal group: Six rats received neither ALX nor taurine and received only water as vehicle. No mortality was found.

Group 2—TAU group: Six rats received only 1% taurine (w/v in water, orally). No mortality was found.

Group 3—ALX group: Thirteen rats received single dose of ALX (Verma, 2010) at a dose of 120 mg/kg body wt in citrate buffer, pH 4.5, i.p. After 3 days of ALX injection, rats having blood glucose level in excess of 300 mg/dL were considered as diabetic. Six rats died during the experimental period.

Group 4—ALX & TAU simultaneous treatment group: Ten rats received taurine (1% w/v in water, orally, 1 h before ALX injection) from the day on which ALX was injected for 21 days. Two rats died during the experimental period.

Group 5—ALX + TAU post treatment group: Ten rats received taurine (1% w/v in water, orally) from the 4th day after ALX injection for 21 days. Three rats died during the experimental period.

Collection of blood, pancreas and heart

Rats in each group were bled every 3 days from the lateral vein of the tail and 100 μL blood was taken for the measurement of plasma glucose.

The experimental rats were euthanized under light ether anesthesia after 3 weeks of treatment with taurine. Pancreases and hearts were removed. Hearts were either stored at −80 °C till biochemical analysis later or fixed in 10% buffered formalin for TUNEL and histological assessments. Pancreases were fixed in 10% buffered formalin for only histological assessments. The body weight and heart weight were measured and compared between groups. Blood samples were drawn from the caudal vena cava. Blood was collected in test tubes containing heparin solution and centrifuged at 1500× g for 10 min to obtain plasma. The plasma was immediately stored at −80 °C until use.

Preparation of nuclear, mitochondrial, cytosolic and membrane fractions

The hearts were minced, washed with saline buffer and homogenized in a Dounce glass homogenizer in homogenizing buffer (50 mM phosphate buffer/1 mM EDTA, pH 7.5, containing 1.5 mM MgCl₂, 10 mM KCl, and supplemented with protease and phosphatase inhibitors). The homogenates were spun down for 10 min at 500× g at 4 °C. The supernatant was collected and recentrifuged at 2000× g for 10 min. The pellet was resuspended in the same buffer and taken as nuclear fraction and stored at −80 °C as described by the method of Lizotte et al. (2009). The supernatant was recentrifuged at 12,000× g for 10 min at 4 °C, and pellet was resuspended in 200 mM mannitol, 50 mM sucrose, 10 mMol/L Hepes-KOH (pH 7.4) and stored as mitochondrial fraction at −80 °C as described by the method of Jang et al. (2004) with some modifications. The final supernatant was taken and centrifuged for 1 h at 40,000× g. The resultant supernatant was used as cytosolic fraction and stored at 4 °C. The pellet was resuspended in the above-mentioned homogenizing buffer, containing 1% (v/v) Triton X-100, by sonication for 7 × 1 s cycles and again centrifuged for 1 h at 40,000× g. The supernatant now contained the extracted membrane fraction and stored at 4 °C (Huisamen et al., 2001).

In the present study, the nuclear fraction has been used for the western blot analysis of Nrf-2; membrane fraction has been used for...
the western blot analysis of GLUT4; mitochondrial fraction has been used for the western blot analysis of cytochrome c and membrane potential determination and finally cytosolic fraction has been used for all other western blot analyses, determination of MDA, protein carbonyl, thiol based antioxidants, xanthine oxidase, ROS, antioxidant enzymes activities, myeloperoxidase activity, IL-6 and TNF-α levels.

Determination of protein content

The protein content of the experimental samples was measured by the method of Bradford (1976) using crystalline BSA as standard.

Biochemical analyses

Plasma glucose and specific markers related to cardiac dysfunction and lipid profiles e.g. LDH, uric acids, total cholesterol and triglyceride levels in the plasma were estimated by using standard kits purchased from Span Diagnostic Ltd., Surat, Gujarat, India. The lipid peroxidation, protein carbonyl content and intracellular ROS were estimated following the method as described by Das et al. (2009a). Activities of antioxidant enzymes (SOD, CAT, GST, GR and GPx) and cellular metabolites levels (GSH and GSSG) in the heart tissue were determined following the method described by Das et al. (2009b). Myeloperoxidase activity was measured by the methods of Naito et al. (1998).

Determination of xanthine oxidase activity in cardiac tissue

Xanthine oxidase activity was assessed by measuring the enzymatic oxidation of xanthine to uric acid following the methods of Bergmeyer et al. (1974). The reaction mixture (3 mL) contained 1.9 mL of 50 mM potassium phosphate buffer, pH 7.5 and 1 mL of 0.15 mM xanthine. The mixture was mixed by inversion and equilibrated at 25 °C. The absorbance was measured at 290 nm until a constant value was obtained. The reaction was started by adding 100 μL of tissue extract and the increase in absorbance was measured at 290 nm for 4 min at 25 °C.

Determination of plasma insulin and cardiac TNF-α and IL-6 levels by ELISA

ELISA was performed by the method of Ausubel et al. (2003). 50 μL antibodies at a concentration of 5 μg/mL were loaded in 96 well plates and incubated at 4 °C overnight. After that, the well plates were washed with wash buffer (25 mM Tris, pH 8.0, 150 mM NaCl and 0.5% Tween 20) twice to remove unbound antibodies. Then 50 μL of plasma/cytosolic protein was added into the wells and incubated at 4 °C overnight. After that, the well plates were washed with wash buffer. Then 50 μL HRP conjugated secondary antibody (200 ng/mL) was added and incubated for 2 h at 4 °C. Then again washing with wash buffer was repeated. The color was developed by adding 100 μL of TMB/H₂O₂ solution and reaction was stopped by adding 2 N H₂SO₄. Then absorbance was measured at 450 nm.

Histological studies

Pancreases and hearts from the normal and experimental rats were fixed in 10% buffered formalin and were processed for paraffin sectioning. Sections of about 5 μm thickness were stained with hematoxylin and eosin to evaluate under light microscope.

Determination of mitochondrial membrane potential (Δψm)

Analytic flow cytometric measurements for the membrane potential (Δψm) of isolated mitochondria were performed using a FACSscan flow cytometer with an argon laser excitation at 488 nm and a 525 nm band pass filter as described by the method of Das et al. (2011a). Mitochondrial membrane potential (Δψm) was measured on the basis of cell retention of the fluorescent cationic probe rhodamine 123.

Immunoblotting in cardiac tissue

Immunoblotting was performed as described by the method of Das et al. (2011a). An equal amount of protein (50 μg) from each sample was resolved by 10% SDS-PAGE and transferred to PVDF membrane. Membranes were blocked at room temperature for 2 h in blocking buffer containing 5% non-fat dry milk to prevent non-specific binding and then incubated with primary antibodies separately at 4 °C overnight; namely, anti-NF-κB (p65) (1:250 dilution, for nuclear fraction), anti GLUT4 (1:1000 dilution, for membrane fraction), anti p-IR (1:1000 dilution), anti p-IRS1 (1:1000 dilution), anti Bad (1:1000 dilution), anti Bax (1:1000 dilution), anti Bcl-2 (1:1000 dilution), anti Bcl-xl (1:1000 dilution), anti caspase3 (1:1000 dilution) and anti Akt (1:100 dilution) for cytosolic fraction and anti cytochrome c (1:1000 dilution) for both cytosolic and mitochondrial fractions. 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).

**DNA fragmentation assay**

Cardiac tissue was washed with STE buffer (0.1 M NaCl, 10 mM Tris–HCl, 1 mM EDTA, pH 8) and 1 ml homogenization buffer (0.1 M NaCl, 0.2 M sucrose, 0.01 M EDTA, 0.3 M tris, pH 8) and 100 μl 10% SDS was added and mixed by vortexing. The mixture was incubated at 65 °C for 1 h. 175 μl of 8(M) potassium acetate was then added and incubated in ice for 1 h, centrifuged and the supernatant was collected. Equal volume of phenol–chloroform mixture was added, mixed and centrifuged to separate phases. The uppermost layer was taken in a fresh tube. Equal volume of chloroform was added and centrifuged. The aqueous layer was taken in a fresh tube. 1/10th vol of 3(M) sodium acetate (pH 7.4) and 2.5 times vol of ethanol were added and centrifuged. The precipitated DNA was washed with 80% ethanol.

The DNA fragmentation has been assayed by electrophoresing genomic DNA samples, isolated as described above from normal as well as experimental rat hearts on agarose/EtBr gel.

**TUNEL staining**

Paraffin embedded cardiac tissue sections (5 μm) were warmed 30 min (64 °C), deparaffinized and rehydrated. Terminal transferase mediated dUTP nick end-labeling of nuclei was performed by using APO-BrdU TUNEL Assay kit (A-23210; Molecular Probes, Eugene, OR) following the manufacturer’s protocol.

**Statistical analysis**

All the values are expressed as mean±S.D. (n = 6). Significant differences between the groups were determined with SPSS 10.0 software (SPSS Inc., Chicago, IL, USA) for Windows using one-way analysis of variance (ANOVA) and the group means were compared by Tukey’s test. A difference was considered significant at the p<0.05 level.

**Results**

**Taurine suppressed the change in body weight, plasma glucose, insulin and cardiotoxicity**

Approximately 85% of the rats injected with ALX developed experimental IDDM, which were characterized by significant body weight (P<0.001) and heart weight (P<0.01) loss (Table 1), increased plasma glucose (P<0.001) (Fig. 2) and decreased plasma insulin levels (P<0.001) (Fig. 3). The rats were behaviorally inactive and mortality rate was approximately 50%. However, treatment with taurine (1%) for 3 weeks recovered the loss of body weight (P<0.001) and heart weight (P<0.01) and increased the survival rate (70–80%) caused by ALX. Taurine treatment also decreased the plasma glucose (P<0.001) and increased the plasma insulin levels (P<0.001, in case of taurine simultaneous treated groups and P<0.01, in case of taurine post treated groups) in diabetic rats. A significant difference was detected in plasma glucose level between taurine simultaneous and taurine post treated groups after three weeks of treatment (P<0.05). Plasma insulin levels were different between normal and taurine simultaneous treated groups (P<0.05) as well as normal and taurine post treated groups (P<0.01).

Results showed that under diabetic condition, the plasma levels of the cardiac damage markers, e.g. LDH (P<0.001) and uric acid (P<0.05) (Table 1) were significantly increased compared to normal and only taurine treated rats. However taurine treatment effectively decreased the LDH (P<0.001) and uric acid (P<0.05) levels compared to diabetic rats.

*Taurine normalized the ALX-induced damage of pancreas*

Histological assessments of various pancreatic segments of the normal and experimental rats were presented in Fig. 4. The pancreatic

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal control</th>
<th>TAU treated</th>
<th>ALX treated</th>
<th>ALX+TAU</th>
<th>ALX+TAU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>202.4±7.12</td>
<td>202.2±7.58</td>
<td>143.6±5.18</td>
<td>198.8±6.94*</td>
<td>197.0±6.91***</td>
</tr>
<tr>
<td>Heart weight (g)</td>
<td>0.58±0.023</td>
<td>0.61±0.115</td>
<td>0.47±0.017**</td>
<td>0.59±0.012**</td>
<td>0.57±0.018***</td>
</tr>
<tr>
<td>Ratio of heart weight to body weight (%)</td>
<td>0.28±0.010</td>
<td>0.31±0.013</td>
<td>0.32±0.015*</td>
<td>0.32±0.012</td>
<td>0.28±0.012</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>151.66±4.58</td>
<td>116.47±3.82*</td>
<td>199.18±7.96**</td>
<td>153.83±5.69***</td>
<td>162.83±6.14***</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.166±0.22</td>
<td>5.808±0.27</td>
<td>10.404±0.32**</td>
<td>5.424±0.17**</td>
<td>5.434±0.17**</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD, for 6 animals in each group.

- Indicates that it differs significantly from normal.
- Indicates that it differs significantly from TAU.
- Indicates that it differs significantly from ALX.

*P<0.05.
**P<0.01.
***P<0.001.

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Fig. 2. Time dependent effect of taurine and diabetes on plasma glucose levels up to 3 weeks. Normal: plasma glucose levels in normal rats; taurine: plasma glucose levels in rats treated with only taurine (1%) for 3 weeks; ALX: plasma glucose levels in rats (closed square) received ALX in a single dose (120 mg/kg body weight, i.p.); ALX+TAU simultaneous (empty circle) and ALX + TAU post (closed circle); plasma glucose levels in diabetic rats treated with taurine. Data represent the average±SD of 6 separate experiments in each group.

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Table 1

Effect of alloxan and taurine on the heart weight, body weight and levels of the serum markers related to cardiac dysfunction of experimental animals.
that it differs significantly from normal; induced diabetic rat heart was tested by measuring lipid peroxidation Taurine suppressed ALX-induced oxidative stress

Islets of Langerhans were smaller with decreased number of β-cells in the ALX-induced diabetic rats than the normal group. However, treatment with taurine prevented the development of ALX-induced changes, thereby suggesting the protective role of taurine in the pancreas of diabetic rats.

Taurine suppressed ALX-induced oxidative stress

In our present study, the occurrence of oxidative stress in ALX-induced diabetic rat heart was tested by measuring lipid peroxidation

It is well known that glucose uptake is controlled by insulin-mediated GLUT4 translocation to the plasma membrane. Therefore, we have investigated whether the hypoglycemic effect of taurine

Taurine normalized the altered lipid profile in diabetic rats

Table 5 showed the effect of taurine on plasma lipid profile in diabetic rats. Under hyperglycemic condition plasma triglyceride (P < 0.001), total cholesterol (P < 0.001), LDL (P < 0.001), LDL/HDL (P < 0.001) and total cholesterol/HDL (P < 0.001) ratios were increased significantly. Taurine significantly lowered the ALX-induced increased triglyceride (P < 0.001), total cholesterol (P < 0.001) and LDL (P < 0.001) levels. However, HDL level was not changed significantly in diabetic rats. Taurine also showed significant decrease in LDL/HDL (P < 0.001) and total cholesterol/HDL (P < 0.001) ratios in diabetic rats. All these results suggested that taurine is effective in maintaining the lipid profile near to that of control in this animal model of diabetes.

Taurine effectively reduced ALX-induced cardiac damage

Fig. 5 showed the histological assessments of cardiac segments of normal, ALX-exposed and taurine-treated animals. Disorganization of the normal radiating pattern of cell plates in the ALX-injected rat hearts was observed. However, treatment with taurine reduced such changes in ALX-injected rat hearts.

Taurine reduced the diabetes-associated up-regulation of NFκB, IL-6, TNF-α and myeloperoxidase activity

NFκB regulates a number of genes (including those coding for key inflammatory cytokines, like IL-6, TNF-α etc.) involved in inflammation. We observed that the protein level of p65 sub-unit of NFκB in nuclear extracts and cardiac levels of the pro-inflammatory cytokines, namely IL-6 (P < 0.001) and TNF-α (P < 0.001) was markedly elevated in diabetic rats compared to normal rats (Fig. 6). Our results also showed that the activity of myeloperoxidase was also increased in diabetic rats (P < 0.001) compared to normal rats (Fig. 4). However, treatment with taurine significantly decreased NFκB expression, IL-6 (P < 0.001) and TNF-α (P < 0.001) levels and myeloperoxidase activity (P < 0.001) compared to diabetic rats. All these results suggested that taurine effectively worked as an anti-inflammatory agent under diabetic conditions.

Taurine improved insulin signaling in cardiac tissue of diabetic rats

It is well known that glucose uptake is controlled by insulin-mediated GLUT4 translocation to the plasma membrane. Therefore, we have investigated whether the hypoglycemic effect of taurine...
Table 2
Effect of alloxan and taurine on the status of the lipid peroxidation and protein carbonylation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal control</th>
<th>TAU treated</th>
<th>ALX treated</th>
<th>ALX&amp;TAU</th>
<th>ALX + TAU</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/mg protein)</td>
<td>1.12±0.03</td>
<td>1.08±0.03</td>
<td>4.36±0.09</td>
<td>2.16±0.06</td>
<td>2.17±0.06</td>
</tr>
<tr>
<td>Protein carbonylation (nmol/mg protein)</td>
<td>14.27±0.614</td>
<td>11.37±0.469</td>
<td>20.02±0.099</td>
<td>14.72±0.614</td>
<td>13.45±0.573</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD, for 6 animals in each group.

a Indicates that it differs significantly from normal.
b Indicates that it differs significantly from TAU.
c Indicates that it differs significantly from ALX.
* P<0.05.
** P<0.01.
*** P<0.001.

was mediated via the insulin-dependent regulation of myocardial GLUT4 translocation and subsequent glucose uptake in ALX-induced diabetic rats. We observed that tyrosine phosphorylation of IRS1, IRS2, and Akt and GLUT4 expression at the membrane fraction were decreased in the heart of diabetic rats, indicating less glucose uptake (Fig. 7). However, taurine treatment significantly increased the ALX-induced reduction of phosphorylation of IR, IRS1, and Akt and increased the membrane translocation of GLUT4. These results showed that taurine enhanced insulin sensitivity in ALX-induced diabetic rats.

Taurine protected against ALX-induced apoptosis in heart

Next we have investigated the mode of cell death in ALX-induced diabetic heart. We observed that ALX significantly increased the pro-apoptotic Bad/Bax and decreased anti-apoptotic Bcl-2/Bcl-xL protein expression, in other words, it disturbed the balance between the pro- and anti-apoptotic Bcl-2 family protein expressions. In addition, ALX also reduced the mitochondrial membrane potential and increased the translocation of cytochrome c from mitochondria (reduced protein expression) into cytosol (increased protein expression) and protein expressions of cleaved caspase 9/3 (Fig. 8). ALX-induced apoptosis was also evident from the ladder pattern (hallmark of apoptosis) obtained in DNA gel electrophoresis (Fig. 8) and TUNEL assay (Fig. 9). As shown in Fig. 9, TUNEL positive nuclear staining was observed in ALX-induced diabetic rat heart indicating apoptosis of the cardiac cells. However, taurine effectively reduced the ALX-induced disturbances in the regulations of Bcl-2 family proteins, cytosolic translocation of cytochrome c, cleavage of caspase 9/3 and number of TUNEL positive nuclei. On the other hand, taurine increased mitochondrial membrane potential and DNA integrity in ALX-exposed rats. All these results clearly demonstrated the anti-apoptotic role of taurine in cardiac tissue under ALX-induced diabetic conditions.

Discussion

Diabetes mellitus is the most common metabolic syndrome and an important health problem worldwide. About 65–70% of diabetic patients (both type 1 and type 2) die as the outcome of heart disease (El-Seweidy et al., 2011). In the present study, we investigated the mechanism of hypoglycemic effect of taurine and its beneficial effect in cardiac damage under diabetic conditions as well. Taurine has been administered orally through drinking water so that it could be better absorbed and in consequence, more effective than routed through added to foods (Winiarska et al., 2009).

ALX, at a dose of 120 mg/kg body weight, caused sufficient damage to pancreatic β cells so that secreted insulin was not enough to regulate blood glucose and resulted to a significant increase in blood glucose levels (Verma et al., 2010). In our experiment, we have shown that taurine markedly decreased plasma glucose levels in ALX-induced diabetic rats via the potent insulin effect by protecting pancreatic β cells. Diabetes is also characterized by severe loss of body weight. The decreased body weight in diabetic rats is due to excessive breakdown of muscle tissue, fats and metabolism of proteins (Chatterjea and Shinde, 2002; Roy et al., 2011). Taurine treatment to diabetic rats, however, showed significant increase in the average body weights compared to the untreated diabetic rats.

Alterations of lipid profile are common in diabetic conditions (Kain et al., 2010). In diabetes, since blood glucose is not utilized by tissue, the fatty acid from adipose tissue are mobilized for energy purpose and excess fatty acids are accumulated in the liver and converted to triglycerides (Shih et al., 1997). Under diabetic condition, cholesterol level was increased due to decreased cholesterol absorption and increased cholesterol biosynthesis (Gylling and Miettinen, 1997). Chronic insulin deficiency is associated with a diminished level of LDL receptor which causes the increase in LDL particles and results to an increase in LDL-cholesterol levels in diabetes mellitus (Suryawanshi et al., 2006). Similar with other studies (Kain et al., 2010; Nizamutdinova et al., 2009), we also observed increased

Table 3
Effect of alloxan and taurine on the status of the thiol based antioxidants, xanthine oxidase activity and intracellular ROS.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal control</th>
<th>TAU treated</th>
<th>ALX treated</th>
<th>ALX&amp;TAU</th>
<th>ALX + TAU</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH (nmol/mg protein)</td>
<td>18.68±0.734</td>
<td>18.44±0.722</td>
<td>16.80±0.640</td>
<td>17.92±0.796</td>
<td>17.98±0.799</td>
</tr>
<tr>
<td>GSSG (nmol/mg protein)</td>
<td>0.64±0.022</td>
<td>0.62±0.021</td>
<td>0.99±0.039a*</td>
<td>0.65±0.022*</td>
<td>0.67±0.024*</td>
</tr>
<tr>
<td>Redox ratio (GSH/GSSG)</td>
<td>29.19±1.359</td>
<td>29.74±1.387</td>
<td>16.92±0.746***</td>
<td>27.56±1.278***</td>
<td>26.84±1.242***</td>
</tr>
<tr>
<td>Xanthine oxidase activity (mIU/mg protein)</td>
<td>0.48±0.032</td>
<td>0.47±0.02650.</td>
<td>0.80±0.055***</td>
<td>0.53±0.0445***</td>
<td>0.55±0.045***</td>
</tr>
<tr>
<td>Intracellular ROS (% over control)</td>
<td>100±4</td>
<td>67±23.5</td>
<td>210±9.5***</td>
<td>102±4.1***</td>
<td>107±4.39***</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SD, for 6 animals in each group.

a Indicates that it differs significantly from normal.
b Indicates that it differs significantly from TAU.
c Indicates that it differs significantly from ALX.
* P<0.05.
** P<0.01.
*** P<0.001.
Table 4
Effect of alloxan and taurine on the activities of the antioxidant enzymes in cardiac tissue.

<table>
<thead>
<tr>
<th>Name of the parameters</th>
<th>Normal control</th>
<th>TAU treated</th>
<th>ALX treated</th>
<th>ALX&amp;TAU</th>
<th>ALX + TAU</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (unit/mg protein)</td>
<td>16.8 ± 0.74</td>
<td>17.4 ± 0.67</td>
<td>5.2 ± 0.16**</td>
<td>14.8 ± 0.64**</td>
<td>15.2 ± 0.66**</td>
</tr>
<tr>
<td>CAT (μmol/min/mg protein)</td>
<td>136.85 ± 5.842</td>
<td>140.25 ± 6.998</td>
<td>83.85 ± 3.193***</td>
<td>134.63 ± 5.732***</td>
<td>125.97 ± 5.298***</td>
</tr>
<tr>
<td>GST (μmol/min/mg protein)</td>
<td>3.66 ± 0.173</td>
<td>3.41 ± 0.169</td>
<td>1.91 ± 0.085***</td>
<td>3.74 ± 0.176***</td>
<td>3.69 ± 0.174***</td>
</tr>
<tr>
<td>GR (nmol/min/mg protein)</td>
<td>6.014 ± 0.299</td>
<td>6.635 ± 0.232</td>
<td>1.792 ± 0.079***</td>
<td>7.835 ± 0.292***</td>
<td>7.659 ± 0.283***</td>
</tr>
<tr>
<td>GPx (nmol/min/mg protein)</td>
<td>119.94 ± 4.79</td>
<td>122.14 ± 4.99</td>
<td>78.41 ± 2.92***</td>
<td>112.43 ± 4.64***</td>
<td>109.20 ± 4.29***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD, for 6 animals in each group.

a Indicates that it differs significantly from normal.
b Indicates that it differs significantly from TAU.
c Indicates that it differs significantly from ALX.
* P<0.05.
** P<0.01.
*** P<0.001.

Table 5
Effect of alloxan and taurine on the serum lipid profile.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal control</th>
<th>TAU treated</th>
<th>ALX treated</th>
<th>ALX&amp;TAU</th>
<th>ALX + TAU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>54.55 ± 1.728</td>
<td>53.58 ± 1.679</td>
<td>100.48 ± 4.024***</td>
<td>56.46 ± 1.823***</td>
<td>61.24 ± 2.062***</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>20.24 ± 0.088</td>
<td>18.32 ± 0.816</td>
<td>17.80 ± 0.69</td>
<td>21.98 ± 0.075</td>
<td>20.24 ± 0.069</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>21.81 ± 1.0805</td>
<td>14.16 ± 0.608</td>
<td>48.83 ± 1.442***</td>
<td>22.71 ± 0.936***</td>
<td>22.72 ± 0.936***</td>
</tr>
<tr>
<td>Total cholesterol/HDL</td>
<td>2.50 ± 0.115</td>
<td>2.92 ± 0.126</td>
<td>5.64 ± 0.182***</td>
<td>2.57 ± 0.119***</td>
<td>3.03 ± 0.102***</td>
</tr>
<tr>
<td>LDL/HDL</td>
<td>1.08 ± 0.044</td>
<td>0.77 ± 0.028***</td>
<td>2.74 ± 0.117***</td>
<td>1.03 ± 0.042***</td>
<td>1.12 ± 0.046***</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>107.83 ± 4.391</td>
<td>105.54 ± 4.277</td>
<td>216.44 ± 9.622***</td>
<td>121.22 ± 5.061***</td>
<td>114.34 ± 4.717***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD, for 6 animals in each group.

a Indicates that it differs significantly from normal.
b Indicates that it differs significantly from TAU.
c Indicates that it differs significantly from ALX.
* P<0.05.
** P<0.01.
*** P<0.001.
of taurine could be directly related to its antioxidative nature which is in agreement with the previous findings (Das et al., 2010a; Ghosh et al., 2009).

Diabetes is also known as an inflammation-prone disease apart from the altered metabolic condition. Hyperglycemia-induced ROS production stimulates the signal transduction pathway for the activation of the transcription factor NF-κB which helps to control the expression of numerous genes activated during inflammation (i.e., cytokines, chemokines, growth factors, immune receptors, cellular ligands, and adhesion molecules) (Kuhad and Chopra, 2009). This leads to cardiac dysfunction and exacerbates the severity of diabetes (Sun et al., 2011). In our present study, augmented cardiac inflammation was observed as evidenced by increased NF-κB nuclear translocation and increased TNF-α and IL-6 levels. Cardiac inflammation was further confirmed by increased myeloperoxidase (MPO) activity. MPO, a major neutrophil protein, is also present in monocytes. It produces hypochlorous acid (HOCl) and other reactive oxidants by using superoxide and hydrogen peroxide generated by the neutrophil oxidative burst. HOCl is a potent mediator of inflammation (Winterbourn et al., 2000). However, being an antioxidant, taurine could effectively antagonize the toxic effect of HOCl by scavenging and forming a stable product, taurine-chloramine which possesses anti-inflammatory and cytoprotective properties (Schuller-Levis and Park, 2003). Besides the inhibition of MPO activity, taurine also decreased the levels of proinflammatory cytokines (TNF-α and
IL-6) and NF-κB nuclear translocation. The inhibition of NF-κB nuclear translocation by taurine has also been reported by other investigators (Giri et al., 2000 and Gurujeyalashmi et al., 2000). Several studies including our own have revealed that taurine is involved in glucose homeostasis, although the specific molecular mechanisms are still unknown. On the other hand, numerous studies have also reported that taurine treatment did not reduce plasma glucose level in diabetic rats (Li et al., 2005; Obrosova et al., 2001). However, this inconsistency might result due to many factors, including the route of taurine administration. It is obvious that, when taurine is supplemented through drinking water, it could be better absorbed and in consequence, more effective than its administration through food stuffs (Winiarska et al., 2009). Several investigators have reported that taurine treatment before diabetic onset suppressed hyperglycemia.

![Fig. 7. Effect of taurine and diabetes on insulin-mediated glucose transport signaling pathways. Panel A: phospho (tyrosine) and total IR, panel B: phospho (tyrosine) and total IRS1, panel C: phospho (serine) and total Akt, and panel D: membrane translocation of GLUT4. Data represent the average ± SD of 6 separate experiments in each group. “a” indicates that it differs significantly from normal; “b” indicates that it differs significantly from TAU; “c” indicates that it differs significantly from ALX; “d” indicates that it differs significantly from ALX&TAU. *P<0.05, **P<0.01.](image)
in streptozotocin-induced type 1 diabetic rats (Alvarado-Vasquez et al., 2003; Tokunaga et al., 1983). On the other hand, it has also been reported that treatment with taurine after diabetic onset failed to suppress hyperglycemia in type 1 diabetic rats, but found to be effective if treated for longer time periods (Di Leo et al., 2004; Vasqueza et al., 2003; Yao et al., 2009). Therefore, the treatment time and routes of administration of taurine are important for its beneficial action in treating diabetes. Several hypotheses concerning the hypoglycemic effect of taurine are: protection of \( \beta \)-cells/\( \beta \)-cell insulin secretion (Chang and Kwon, 2000; Cherif et al., 1998; Gavrovskaya et al., 2008; Kaplan et al., 2004); enhanced insulin sensitivity (Carneiroa et al., 2009; Colivicchi et al., 2004; Wu et al., 2010), insulin like effect (Kulakowski and Maturo, 1984; Maturo and Kulakowski, 1988), diminished glucose absorption from gastrointestinal tract (Kim et al., 2006) and its accelerated utilization by peripheral tissues (Nandhini et al., 2004) although there is no earlier report concerning the insulin sensitivity or insulin mimetic effect of taurine in ALX-induced type I diabetic rats. Like other tissues, insulin signaling via
Akt/GLUT4 pathways plays a key role in cardiac glucose uptake (Brownsey et al., 1997). Cardiac tissues obtain its energy by the oxidation of fatty acids, glucose, lactate and ketone bodies (Rodrigues and McNeill, 1992). During diabetes or insulin resistance, the energy metabolism is altered in heart due to less glucose uptake; as a result glucose utilization is decreased and fatty acid consumption is increased. This disturbance of glucose metabolism worsen metabolic efficiency of cardiomyocytes and plays an important role in the development of atherosclerotic heart disease, left ventricular hypertrophy and dysfunction, heart failure and cardiomyopathy (Murakami et al., 2004). In our present study, we observed that under diabetic conditions, phosphorylation at tyrosine residue of IR and IRS1 proteins; and phosphorylation at serine residue of Akt protein were decreased. We also observed decreased GLUT4 expressions in diabetic hearts. However, administration of taurine to diabetic rats significantly improved this insulin signaling pathway and restored GLUT4 translocation to the plasma membranes for the uptake of glucose. These results are also supported by previous findings that taurine may act directly on the insulin receptor to facilitate glucose uptake (Kulakowski and Maturo, 1984; Maturo and Kulakowski, 1988) and suggest that taurine seems to be beneficial to decrease diabetes-associated cardiac complications via increased glucose uptake in the heart.

In the present study, we have also investigated the involvement of ALX-mediated intrinsic apoptotic cell death pathway in cardiac tissues. Immunoblot analyses revealed that taurine treatment protected ALX-induced apoptotic cell death in cardiac tissues via the regulation of pro-apoptotic proteins, such as Bax/Bad and anti-
apoptotic proteins, such as Bcl-2/Bcl-xl, lowering of mitochondrial membrane potential, enhancing the release of cytochrome c into cytosol and the cleavage of caspase-9/3. In addition, ALX-induced cardiac apoptotic cell death was also evident from DNA fragmentation and TUNEL assays. However, taurine treatment protected cardiac tissue from ALX-induced DNA breakdown and decreased the number of TUNEL positive nuclei.

In conclusion, the results of the present study demonstrates that (i) taurine administration exerts anti-hyperglycemic effects, controls blood glucose levels and restores body weight in diabetic animals; (ii) taurine increases the level of insulin in diabetic rats (this effect is more pronounced in taurine simultaneous treated group compared to taurine post treatment); (iii) taurine restores GLUT4 expression in the plasma membranes of the heart tissues via insulin dependent mechanism (this effect is more pronounced in taurine post treated group compared to taurine simultaneous treated group), thereby inducing glucose uptake in diabetic conditions; (iv) taurine exerts antioxidant, antiinflammatory and antiapoptotic activities and (v) taurine protects cardiac tissue against apoptosis induced by ALX as indicated in the proposed scheme (Fig. 10). Taken together, our results suggest that taurine supplementation may be considered as an applicable approach in preventing or treating diabetes and associated cardiac complications.

Conflict of interest
The authors declare that no conflict of interest exists.

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References


