CHAPTER - 4

AMELIORATION OF RENAL OXIDATIVE STRESS BY NIGERLOXIN IN INDUCED NEPHROPATHY
**Section - A**

**AMELIORATION OF NEPHROPATHY BY NIGERLOXIN IN STREPTOZOTOXIN INDUCED DIABETIC RATS**

**Introduction**

Nephropathy is one of the secondary complications that diabetic patients are at higher risk of developing. Diabetic nephropathy usually develops in 30-40% of insulin depended diabetes mellitus patients and terminal renal failure occurs within 7 years after the onset of renal disease. It is widely accepted that hyperglycemia plays major role in the pathogenesis and progression diabetic complications including nephropathy. Several biochemical mechanisms have emerged to explain the adverse influence of hyperglycemia. Among these, polyol pathway (Oates and Mylari, 1999; Schrijvers et al. 2004) and oxidative stress (Wolff et al. 1991; Ha and Kim, 1995; Baynes and Thorpe, 1999; Ceriello, 2000) have been suggested extensively as potential mechanisms for the development of microvascular complications, including early diabetic nephropathy.

Aldose reductase (ALR), a key enzyme in polyol pathway converts glucose to sorbitol, resulting in the accumulation of sorbitol in the cells and thereby causes organ injuries by imparting high osmotic pressure (Greene et al. 1987). Oxidative stress which results from an imbalance between the generation of reactive oxygen species (ROS) and the organism’s antioxidant potential also play major role in diabetic complication. ROS are produced through several mechanisms in hyperglycemic condition. Mainly an excess of intracellular glucose increases superoxide radical formation in the mitochondria at the electron transport chain (Brownlee, 2001). Another source of free radicals in diabetes is the nonenzymatic interaction of glucose with proteins leading to the formation of an Amodori product and then advanced glycation endproducts (Mullarkey et al. 1990; Hori et al. 1996).
In hyperglycemic conditions, increased intracellular concentrations of ROS appear to overcome the ability of many cells to neutralize radicals. An altered activity of antioxidant enzymes in the kidney of streptozotocin (STZ)-induced diabetic rats has been reported (Kashihara et al. 2010). Antioxidant defenses are further depleted by decreased level of glutathione, a major antioxidant molecule, due to over-utilization of reduced nicotinamide coenzymes in polyol pathway. Damage from polyol pathway, ROS and depleted cellular antioxidant defenses manifested in kidney results in progression of end-stage renal disease.

Inhibition of enzyme ALR and potentiating antioxidant defense system may prevent or arrest the progression of long-term diabetic complications. Several animal studies have reported a favorable effect of aldose reductase inhibitors (ARIs) (Bank et al. 1989; Tilton et al. 1989; McCaleb et al. 1991; Isogai et al. 1995) and antioxidants on diabetic nephropathy (Melhem et al. 2001; Melhem et al. 2002). But none have achieved clinical success for diverse reasons. Hence search for newer molecules to treat diabetic complication has become an important pharmaceutical goal. A new aldose reductase inhibitor molecule, viz., nigerloxin, 2-amido-3-hydroxy-6-methoxy-5-methyl-4-(prop-1'-enyly) benzoic acid obtained from solid-state fermentation of Aspergillus niger CFR-W-105 exhibited inhibitory activity on the partially purified ALR and a free radical scavenging activity in vitro (Rao et al. 2002; Rao et al. 2005). The present animal study was carried out to verify if this fungal metaboite exhibits ALR inhibitory activity in vivo in diabetic kidney and to further examine the influence of nigerloxin on modulation of the renal injuries associated with diabetes in experimental rats, by virtue of its ALR inhibitory potential.

Materials and methods

Chemicals

Streptozotocin, glucose oxidase, horse raddish peroxidase, o-dianisidine, nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH), nicotinamide adenine dinucleotide (reduced form) (NADH), adenosine diphosphate (ADP), ascorbic acid,
thiobarbituric acid, glyceraldehyde, reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase, cytochrome-C, xanthine oxidase, hydrogen peroxide, tert-butyl hydroperoxide (TBHP), 1-chloro-2,4-dinitrobenzene (CDNB), 5,5'-dithiobis-2-nitrobenzoate (DTNB), ethylene diamine tetraacetic acid (EDTA), fructose, tetramethoxy propane (TMP) and bovine serum albumin (BSA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade obtained from SISCO Research Laboratories (Mumbai, India).

**Production of nigerloxin**

Nigerloxin was obtained through solid state fermentation of *Aspergillus niger* (Rao et al. 2002; Rao et al. 2005) as described earlier in Chapter-2A. The purity of nigerloxin was confirmed by high performance liquid chromatography using a Shimadzu system (Model LC 10AVP, Shimadzu Corporation, Kyoto, Japan) (Rao et al. 2002).

**Animal treatment**

The animal study was carried out taking appropriate measures to minimize pain or discomfort and with due approval from the Institutional Animal Ethics Committee of this Institute. Male Wistar rats weighing 140–150 g raised by Experimental Animal Production Facility of CFTRI were used in this investigation. Experimental diabetes was induced by a single *i.p.* injection of streptozotocin to animals fasted overnight at a dose of 50 mg/kg body weight in freshly prepared 0.1M citrate buffer (pH 4.5) and the control rats received 0.1M citrate buffer (pH 4.5) alone. Blood samples were obtained from retro orbital plexus at 72 h after an over night fast. Fasting blood glucose levels were determined by glucose oxidase method (Huggett and Nixon, 1957). Rats with fasting blood glucose levels above 250 mg/dL were sorted and used as diabetic animals.

Three groups of diabetic animals (*n* = 8 per group) and a parallel 2 groups of normal animals (*n* = 8 per group) were maintained on semisynthetic basal diet which consisted of the following ingredients (*w/w*): casein (21%), cane sugar (10%), corn starch (54%), refined peanut oil (10%), NRC vitamin mixture (1%) and Bernhart-Tommarelli modified
salt mixture (4%). Experimental animals were housed individually in stainless steel cages under standard laboratory conditions at room temperature with 12 h light and dark cycles. All animals had free access to food and water. Nigerloxin was administered to one group of diabetic rats at a dose of 25 mg/kg body weight and to another group of diabetic rats at a dose of 100 mg/kg body weight as 0.5 mL solution in 3% aqueous starch orally once a day for 30 days. One group of normal rats also received nigerloxin at a dose of 100 mg/kg body weight orally once a day for 30 days. Parallel groups of diabetic rats and normal rats which served as control animals received daily administration of the vehicle alone. 24 h urine samples were collected under toluene at the end of each week of the experimental period.

At the end of the experiment, the animals were weighed, fasted overnight and sacrificed over light ether anaesthesia. Blood was collected in heparinised tubes by cardiac puncture and plasma was separated by centrifugation and stored at –40 °C until further analysis. Kidneys were quickly excised, weighed and processed for further analysis. A 10% (w/v) kidney homogenate was prepared in 50 mM phosphate buffer (pH 7.4) and centrifuged at 15,000 x g at 4 °C for 15 min. All the biochemical parameters were analyzed in the soluble fraction of kidney homogenate.

**Glucose, urea, creatinine and protein**

Plasma and urinary glucose level were determined by glucose oxidase peroxidase method of Huggett and Nixon, (1957). Urinary protein was measured by the Bradford’s dye binding method (Bradford, 1976). Urinary urea was assayed by the method of Wybenga et al. (1971), while creatinine was measured by the method of Folin and Wu (Oser, 1965). Total protein in kidney homogenate was determined by Lowry’s method using BSA as standard (Lowry et al. 1951).

**Lipid peroxides in kidney**

Kidney lipid peroxides were assayed by measuring the malondialdehyde (MDA) concentration as thiobarbituric acid reactive substances (TBARS) fluorimetrically
according to the method described by Ohkawa et al. (1979). The intensity of emitted fluorescence of the extracted butanol was recorded at 553 nm by excitation at wavelength 515 nm and was compared with the standard TMP.

**Kidney advanced glycation end products related and tryptophan fluorescence**

Advanced glycation end products (AGEs) related fluorescence spectra were obtained from 400–500 nm with excitation at 370 nm in a spectrofluorometer (Schimadzu RF-50301 PC; Shimadzu Corporation, Kyoto, Japan) (Monnier and Cerami, 1981) and tryptophan fluorescence spectra were obtained at excitation 295 nm and emission between 310–400 nm.

**Antioxidant molecules**

Reduced glutathione (GSH) in kidney was estimated according to the protocol described by Beutler et al. (1963). Total thiols in kidney homogenates were determined spectrometrically as described by Sedlak and Lindsay, (1968). Ascorbic acid was measured spectrophotometrically according to the method given by Omaye et al. (1973).

**Antioxidant enzymes in kidney**

Catalase activity was assayed by following the rate of decomposition of hydrogen peroxide as described by Aebi, (1984). Superoxide dismutase activity was measured by quantifying the inhibition of cytochrome-C reduction in xanthine–xanthine oxidase system as described by Flohe and Otting, (1984). Glutathione peroxidase activity was measured as described by Flohe and Gunzler, (1984). Glutathione reductase activity was assayed by measuring the oxidation of NADPH by oxidized glutathione according to Carlberg and Mannervik, (1985). Glutathione-S-transferase activity was assayed using the CDNB and by measuring the CDNB-GSH complex formed as described by Warholm et al. (1985).

**Kidney polyol pathway enzymes**

Aldose reductase activity was measured spectrophotometrically according to Kim and Oh, (1999). The assay mixture contained 0.32 mM NADPH and enzyme source in
phosphate buffer (0.135 M; pH 7.0). The reaction was initiated by the addition of 5.5 mM DL-glyceraldehyde and monitored by following the decrease in absorbance at 340 nm. Sorbitol dehydrogenase activity was measured using fructose as substrate according to Gerlach and Hiby, (1974).

Statistical analysis

All analytical data are expressed as mean ± standard error of mean (SEM). Significance of difference between the groups was evaluated by analysis of variance (ANOVA) followed by Dunnett multiple comparisons test. $P<0.05$ was considered statistically significant.

Results

Influence of nigerloxin on hyperglycemia, and urinary excretion of metabolites

Diabetic rats were characterized by markedly higher plasma glucose levels, the initial levels being more than 3-times those of normal animals. Plasma glucose levels did not alter in diabetic animals treated for 30 days with nigerloxin at either 25 mg/kg or 100 mg/kg as compared to untreated diabetic rats (Data not shown). Urinary excretion of glucose, urea, protein and creatinine in diabetic rats administered nigerloxin is presented in Fig.1. The absence of any beneficial modulation of nigerloxin on the hyperglycemic status is also corroborated by data on urinary glucose excretion. Similarly, nigerloxin did not have any beneficial influence on the urinary excretion of urea, protein and creatinine.

Influence of nigerloxin on kidney polyol pathway enzymes

The activities of kidney polyol pathway enzymes, aldose reductase and sorbitol dehydrogenase are presented in Table-1. Renal polyol pathway enzyme activities were significantly increased in diabetic rats. Aldose reductase activity was higher by 55%, while sorbitol dehydrogenase activity was 33% higher in diabetic rats compared to normal controls. Nigerloxin administration at 100 mg/kg body weight significantly countered this increase in activities of these enzymes in diabetic rats. The extent of decrease in the activities of kidney aldose reductase was 24% while that of sorbitol
dehydrogenase was 10%. The lower dose of nigerloxin did not produce any appreciable decrease in the same. Nigerloxin did not have any effect on the activities of polyol pathway enzymes in normal rats.

**Influence of nigerloxin on kidney AGES and tryptophan fluorescence**

Kidney AGES and tryptophan related fluorescence intensity data are presented in Fig.2. AGES related fluorescence of kidney protein was significantly increased in diabetic animals (Fig.2A). Nigerloxin administration especially at the higher dose countered the formation of AGES in diabetic kidney. On the other hand, tryptophan related fluorescence decreased markedly in the kidney of diabetic rats (Fig.2B). Nigerloxin administration especially at the higher dose countered this alteration of protein in kidney of diabetic animals.

**Influence of nigerloxin on kidney lipid peroxides**

Diabetic kidney is characterized by hypertrophy and the weight of kidney was 2.85-fold compared to the normal weight. Administration of nigerloxin did not have any influence on the increasing kidney weight in diabetic rats (Table-2). Influence of nigerloxin on kidney MDA measured as TBARS levels is presented in Table-2. Lipid peroxide level in the kidney of diabetic rat was significantly increased, indicating an elevated lipid peroxidation due to hyperglycemia. Oral administration of nigerloxin for 30 days significantly (p<0.05) countered increase in lipid peroxidation and the decrease was by 24% as compared to diabetic animals. On the other hand, nigerloxin did not produce any significant changes in normal animals.

**Influence of nigerloxin on kidney antioxidant molecules**

The influence of nigerloxin on renal antioxidant molecules in diabetic animals is summarised in Table-2. There was a significant decrease in the concentration of GSH, a major antioxidant molecule in kidney of diabetic rats when compared with the normal control animals. The decreased renal glutathione content of diabetic rats was completely restored by the daily administration of nigerloxin at 100 mg/kg body weight. Ascorbic
Fig. 1. Urinary excretion of glucose, urea, protein and creatinine in diabetic rats treated with nigerloxin.

Values are mean ± SEM of 8 animals in each group.
**Table-1.** Influence of nigerloxin on polyol pathway enzymes in the kidney of diabetic rats.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Aldose Reductase (mmol/min/100 mg protein)</th>
<th>Sorbitol Dehydrogenase (mmol/min/100 mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.055 ± 0.001</td>
<td>1.54 ± 0.030</td>
</tr>
<tr>
<td>Normal + Nigerloxin (100 mg/kg)</td>
<td>0.058 ± 0.002</td>
<td>1.62 ± 0.044</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>0.085 ± 0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.05 ± 0.044&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + Nigerloxin (25 mg/kg)</td>
<td>0.081 ± 0.003</td>
<td>1.94 ± 0.078</td>
</tr>
<tr>
<td>Diabetic + Nigerloxin (100 mg/kg)</td>
<td>0.065 ± 0.002&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.85 ± 0.024&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values are mean ± SEM of 8 animals in each group.

a: Significantly ($P<0.05$) different from Normal Control group.
b: Significantly ($P<0.05$) different from Diabetic Control group.
Fig. 2. Influence of nigerloxin on: (A) Advanced glycation end products related fluorescence and (B) tryptophan fluorescence in diabetic rat kidney.
acid was decreased in the kidney of diabetic rats by as much as 50%. Administration of nigerloxin tended to significantly ($p<0.05$) elevate (by 69%) this antioxidant molecule in the kidney of diabetic rats. The decreased renal total thiol content of diabetic rats was completely restored by the daily administration of nigerloxin at 100 mg/kg body weight.

**Influence of nigerloxin on kidney antioxidant enzymes**

The influence of daily administration of nigerloxin on the activities of renal antioxidant enzymes are presented in Table-3. Activities of renal antioxidant enzymes – superoxide dismutase, glutathione reductase, glutathione-S-transferase and catalase were significantly decreased in diabetic situation. The only exception was that of glutathione peroxidase activity which was slightly increased compared to normal animals. Nigerloxin treatment especially at the daily higher dose of 100 mg/kg body weight significantly ($p<0.05$) countered this fall in renal antioxidant enzyme activities. The increase in the activities brought about by nigerloxin was superoxide dismutase: 14%, catalase: 43%, glutathione-S-transferase: 95%, and glutathione reductase: 48%.
Table-2. Influence of nigerloxin on kidney antioxidant molecules in diabetic rats.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Kidney weight (g/100 g body weight)</th>
<th>Kidney lipid peroxides (µmol/mg protein)</th>
<th>Ascorbic acid (µg/mg protein)</th>
<th>Total thiols (mM/mg protein)</th>
<th>Reduced glutathione (µg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>0.81 ± 0.03</td>
<td>1.15 ± 0.048</td>
<td>1.524 ± 0.099</td>
<td>0.266 ± 0.012</td>
<td>0.113 ± 0.002</td>
</tr>
<tr>
<td>Normal + Nigerloxin (100 mg/kg)</td>
<td>0.86 ± 0.04</td>
<td>1.19 ± 0.071</td>
<td>1.435 ± 0.086</td>
<td>0.275 ± 0.017</td>
<td>0.111 ± 0.003</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>2.31 ± 0.09</td>
<td>1.62 ± 0.113&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.774 ± 0.033&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.213 ± 0.007&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.097 ± 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + Nigerloxin (25 mg/kg)</td>
<td>2.32 ± 0.13</td>
<td>1.54 ± 0.175</td>
<td>0.657 ± 0.129</td>
<td>0.235 ± 0.011</td>
<td>0.101 ± 0.003</td>
</tr>
<tr>
<td>Diabetic + Nigerloxin (100 mg/kg)</td>
<td>2.40 ± 0.22</td>
<td>1.23 ± 0.058&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.310 ± 0.043&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.269 ± 0.011&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.110 ± 0.002&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values are mean ± SEM of 8 animals in each group.

a: Significantly ($P<0.05$) different from Normal Control group.
b: Significantly ($P<0.05$) different from Diabetic Control group.
Table 3. Influence of nigerloxin on kidney antioxidant enzymes in diabetic rats.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Superoxide dismutase (U/mg protein)</th>
<th>Glutathione–S-transferase (mmol/min/mg protein)</th>
<th>Glutathione Reductase (mmol/min/mg protein)</th>
<th>Glutathione peroxidase (mmol/min/mg protein)</th>
<th>Catalase (mmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>24.8 ± 0.36</td>
<td>0.105 ± 0.004</td>
<td>0.043 ± 0.002</td>
<td>0.105 ± 0.005</td>
<td>0.048 ± 0.002</td>
</tr>
<tr>
<td>Normal + Nigerloxin (100 mg/kg)</td>
<td>22.5 ± 0.53</td>
<td>0.097 ± 0.005</td>
<td>0.045 ± 0.002</td>
<td>0.092 ± 0.004</td>
<td>0.044 ± 0.003</td>
</tr>
<tr>
<td>Diabetic Control</td>
<td>19.8 ± 0.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.039 ± 0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.029 ± 0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.122 ± 0.006&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.023 ± 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic + Nigerloxin (25 mg/kg)</td>
<td>22.2 ± 0.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.059 ± 0.003&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.032 ± 0.001</td>
<td>0.127 ± 0.006</td>
<td>0.021 ± 0.002</td>
</tr>
<tr>
<td>Diabetic + Nigerloxin (100 mg/kg)</td>
<td>22.5 ± 0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.076 ± 0.004&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.043 ± 0.003&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.130 ± 0.004</td>
<td>0.033 ± 0.003&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

All values are mean ± SEM of 8 animals in each group.

a: Significantly (<i>P</i>&lt;0.05) different from Normal Control group.
b: Significantly (<i>P</i>&lt;0.05) different from Diabetic Control group.
Discussion

Diabetic nephropathy is a leading cause of end-stage renal failure worldwide. Its morphologic characteristics include glomerular hypertrophy, basement membrane thickening, mesangial expansion, tubular atrophy, interstitial fibrosis and arteriolar thickening, all of which are part of microvascular complications of diabetes (Kashihihara et al. 2010). A large body of evidence indicates that oxidative stress is the common factor in the development and progression of diabetic micro- as well as macro-vascular complications of diabetes. AGEs, defects in polyol pathway, uncoupled nitric oxide synthase (NOS) and NAD(P)H oxidase have been implicated for increased generation of ROS. The present study indicated that nigerloxin possesses a significant favorable effect on antioxidant defense system in the kidney of diabetic animals. A large amount of data emphasizes the role of polyol pathway in the development of diabetic complications. Hyperglycemia accelerates the activity of ALR, a key enzyme in the polyol pathway resulting in an increased enzymatic conversion of glucose to sorbitol with concomitant decrease in NADPH and glutathione (Brownlee, 2001). The loss of antioxidant reducing equivalents results in enhanced sensitivity to oxidative stress associated with intracellular ROS. Intracellular accumulation of sorbitol can result in an increased intracellular osmotic pressure and depletion of myo-inositol leading to cellular swelling, decreased activity of ATPase, and increased cellular membrane permeability. The same changes have been seen in the kidney of diabetic rats. Increased ALR activity triggers signal transduction pathways such as protein kinase C (PKC), P38 mitogen-activated protein kinases and c-Jun N-terminal kinases, which result in overproduction of cytokines such as transforming growth factor-beta and Tumor necrosis factor-alpha leading to some of the pathophysiological changes associated with diabetic nephropathy (Price et al. 2004; Li et al. 2002; Chaturvedi et al. 2002). Additionally, sorbitol dehydrogenase oxidizes sorbitol to fructose resulting in an increased ratio of NADH/NAD and contributes to fructosylation of proteins via providing 3-deoxy glucosone, the precursor to AGEs (Niwa, 1999). Several studies have reported elevation of ALR activity in renal tissue (Ghahary et al. 1989) and favorable influence of ARIs in
diabetic nephropathy (Oates and Mylari, 1999; Schrijvers et al. 2004). In our present study, both aldose reductase and sorbitol dehydrogenase activities were significantly increased in diabetic animals. Nigerloxin treatment significantly inhibited this elevation in aldose reductase and sorbitol dehydrogenase activities in diabetic animals. Thus, the in vivo potential of nigerloxin to inhibit the enzyme aldose reductase is incidentally verified in this animal study.

Increasing evidence in both preclinical and clinical studies suggest that oxidative stress play a major role in the pathogenesis of diabetic complications. Hyperglycemia induces free radical generation leading to lipid peroxidation, a major marker of oxidative stress. In our experimental model, there was a marked increase in TBARS levels which is an index of lipid peroxidation in the kidney of diabetic rats. It has been reported that kidney MDA levels of STZ-induced diabetic rats are increased by lipid peroxidation (Kedziora-Kornatowska et al. 2003). Administration of nigerloxin significantly decreased the level of TBARS of kidney in diabetic rats.

Hyperglycemia derived free radicals impairs the endogenous antioxidant defense system in many ways during diabetes. Antioxidant defense mechanism involves enzymatic and non-enzymatic strategies. The major enzymes of antioxidant defense include superoxide dismutase, catalase, glutathione-S-transferase, glutathione reductase and glutathione peroxidase. Reduced activities of superoxide dismutase, catalase, glutathione-S-transferase and glutathione reductase in kidney have been observed in diabetic animals in the present study. The depletion in the activities of these enzymes augments the deleterious influence of oxidative stress in diabetes. Superoxide dismutase is an important enzyme which converts superoxide anion radicals to hydrogen peroxide, thereby reducing the formation of highly reactive peroxynitrite radical with nitric oxide. The reduction in the activity of this enzyme may result in a number of deleterious effects due to the accumulation of superoxide anion radicals. Catalase protects tissues against hydroxyl radicals by decomposing hydrogen peroxide to water and oxygen. Glutathione-S-transferase catalyses the reduction of hydrogen peroxide and hydro peroxide to non-
toxic products. Glutathione peroxidase metabolizes hydrogen peroxide to water by using reduced glutathione as a hydrogen donor and glutathione disulfide is recycled back to glutathione by glutathione reductase using the cofactor NADPH.

Daily administration of nigerloxin at the higher dose of 100 mg/kg body weight countered the decrease in activities of superoxide dismutase, catalase, glutathione-S-transferase and glutathione reductase in the kidney of diabetic rats. In this context, other investigators also have reported a decrease in the activities of antioxidant enzymes in liver and kidney of diabetic rats (Kashihara et al. 2010).

A decline in major nonenzymatic antioxidant molecules including ascorbic acid, GSH and total thiols markedly increases susceptibility to oxidative stress in diabetes. Ascorbic acid is a powerful inhibitor of lipid peroxidation and it scavenges aqueous superoxide radicals. GSH is the most important biomolecule participating in the elimination of hydroperoxides in the presence of glutathione peroxidase. GSH also functions as free radical scavenger and in the repair of radical mediated biological damage (Meister, 1984; Nicotera and Orrenius, 1986). Numerous studies have revealed lowered antioxidant and enhanced peroxidative status in diabetes condition (Sinclair, 1993). In the present study, we observed a significant reduction in nonenzymatic antioxidant defense molecules like ascorbic acid, GSH, and total thiols in the kidney of diabetic rats. Nigerloxin was found to possess antioxidant potential by significantly countering the depletion of antioxidant molecules in the kidney in diabetic situation.

Chronic hyperglycemia leads accumulation of AGEs in kidney, resulting in increased membrane permeability and loss of integrity. Since AGEs play a role in the development of diabetic nephropathy (Cooper, 2001), we also measured the AGE related fluorescence in the kidney of diabetic rats. Nigerloxin treatment especially at the higher dose of 100 mg/kg body weight significantly inhibited the formation of AGEs in the kidney of diabetic animals.
In conclusions, the present animal study has evidenced that uncontrolled diabetes in rats is associated with enhanced lipid peroxidation, AGEs formation, elevated activity of polyol pathway enzymes and compromised antioxidant defense system in kidney which would promote diabetic nephropathy. Our experimental data suggest that while daily administration of nigerloxin did not have any influence on the hyperglycemic status, urinary excretion of diabetes related metabolites, and kidney weight, the same significantly countered lipid peroxidation and AGEs formation in the renal tissue of diabetic rats. The results also suggested the beneficial influence of nigerloxin on the elevated polyol pathway enzymes and potentiation of antioxidant defense system in diabetic animals. These data suggest that nigerloxin have the potential role in ameliorating oxidative stress in diabetic nephropathy. This is the first report on the potential of this fungal metabolite in the management of diabetic complications, particularly to reduce reno-vascular complications of diabetes.

Summary

Elevated polyol pathway enzyme activities and oxidative stress play an important role in the development and progression of diabetic nephropathy. Here, we investigated the beneficial influence of nigerloxin, a fungal metabolite and a potent aldose reductase inhibitor and free radical scavenger in the kidney of streptozotocin-induced diabetic rats. A group of diabetic rats was orally administered with nigerloxin for 30 days (100 mg/kg). Diabetic rats showed increased lipid peroxides, advanced glycation end products (AGEs), elevated activities of polyol pathway enzymes and lowered antioxidant defense system in kidney. Administration of nigerloxin decreased kidney lipid peroxides and AGEs. Activities of polyol pathway enzymes were reduced while activities of antioxidant enzymes, glutathione and ascorbic acid were elevated in the kidney of nigerloxin treated diabetic rats. The results indicated the beneficial influence of nigerloxin on polyol pathway and oxidative stress associated with diabetes which are implicated in ameliorating the development of diabetic nephropathy.
AMELIORATION OF GENTAMICIN-INDUCED RENAL OXIDATIVE STRESS BY NIGERLOXIN IN RATS

Introduction

Gentamicin is a widely used aminoglycoside antibiotic (Fig.1) in the treatment of severe gram negative bacterial infection. Nephrotoxicity is the common adverse effect of gentamicin treatment that markedly limits its use. It is reported that 30% of the patients treated with gentamicin for more 7 days show signs of nephrotoxicity (Cuzzocrea et al. 2002). Gentamicin nephrotoxicity is typically characterized by tubular damage arising from tubular epithelial cell cytotoxicity. Treatment of experimental animals with gentamicin produces apoptosis (Li et al. 2009) as well as necrosis (Edwards et al. 2007) of tubular epithelial cells in vivo and also in cultured cells (Pessoa et al. 2009). The exact mechanism by which gentamicin induces nephrotoxicity is unknown. It is well documented that reactive oxygen species (ROS) are involved in this (Cuzzocrea et al. 2002; Mazzon et al. 2001; Morales et al. 2002). Studies have demonstrated that gentamicin produces oxidative stress in tubular cells, both in vivo in rats (Karatas et al. 2004) and in cultured tubular cells (Juan et al. 2007). This oxidative stress is mediated by hydroxyl radicals from hydrogen peroxide and by superoxide anions (Basnakian et al. 2002).

Enhanced production of ROS (Morales et al. 2002; Yanagida et al. 2004), causing deficiency in intrinsic antioxidant enzymes (Maldonado et al. 2003) and molecules leads to undesired consequences of oxidative stress. ROS have been suggested as a cause of death for many cells in different pathological states including various models of renal and cardiac diseases (Baliga et al. 1999). It is reported that renal cortical lipoperoxidation (Cuzzocrea et al. 2002; Mazzon et al. 2001; Abdel-Naim et al. 1999; Ali, 2002) and in vivo renal hydrogen peroxide generation (Guidet and Shah, 1989) are increased and glutathione (GSH) is decreased (Ali, 2002; Sener et al. 2002).
Fig. 1. Structure of gentamicin
Antioxidants have been shown to diminish the gentamicin-induced nephropathy in animal models (Yanagida et al. 2004; Maldonado et al. 2003; Pedraza-Chaverri et al. 2004; Koyner et al. 2008). We have recently reported (Suresha et al. 2012) the anti-cataractogenic influence of nigerloxin, a fungal metabolite 2-amido-3-hydroxy-6-methoxy-5-methyl-4-(propenyl) benzoic acid, a known free radical scavenger and aldose reductase inhibitor (Rao et al. 2002; Rao et al. 2005), obtained from solid-state fermentation of *Aspergillus niger* CFR-W-105. We have also observed significant amelioration of nephropathy by administration of nigerloxin in streptozotocin induced diabetic rats (Chapter-4A). In the present investigation, we examined the beneficial influence of nigerloxin, for a possible beneficial modulation of the renal injuries induced with gentamicin in experimental animals.

**Material and methods**

**Chemicals**

Gentamicin (Genticyn, 80 mg/2 mL) from Abbott Healthcare Pvt Ltd. Nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH), adenosine-5’-diphosphate (ADP), thiobarbituric acid, hydrogen peroxide (H$_2$O$_2$), reduced glutathione (GSH), oxidized glutathione (GSSG), glutathione reductase, cytochrome-C, xanthine, xanthine oxidase, tert-butyl hydroperoxide (TBHP), 1-chloro-2,4-dinitrobenzene (CDNB), 5,5’-dithiobis-2-nitrobenzoate (DTNB), ethylene diamine tetraacetic acid (EDTA), ascorbic acid, tetramethoxy propane (TMP), creatinine, urea, bovine serum albumin (BSA) were obtained from Sigma Aldrich Co. (St. Lousis, MO, USA). All other chemicals and solvents used were of analytical grade obtained from SISCO Research Laboratories (Mumbai, India).

**Production of Nigerloxin**

Nigerloxin was obtained through solid state fermentation of *Aspergillus niger* (Rao et al. 2002; Rao et al. 2005), as described by us previously (Chapter-2A; Suresha et al. 2012). The purity of nigerloxin was confirmed by HPLC using a Shimadzu system (Model LC 10AVP, Shimadzu Corporation, Kyoto, Japan) (Rao et al. 2002).
Animal treatment

Male Wistar rats initially weighing 170–175 g raised by Experimental Animal Production Facility Unit of this institute were used in this investigation with due approval from the Institute’s Animal Ethics Committee. Experimental animals were housed in stainless steel cages under standard laboratory conditions at room temperature with 12 h light and dark cycles. Animals were randomly divided in five groups of 8 each as follows; (1) Control, treated with vehicle intraperitoneally, (2) Nigerloxin (100 mg/kg), treated with vehicle intraperitoneally and nigerloxin at a dose of 100 mg/kg body weight, orally for 8 days, (3) Gentamicin, treated with gentamycin at a dose of 80 mg/kg body weight, i.p., for a period of 8 days, (4) Gentamicin + Nigerloxin (25 mg/kg), treated with nigerloxin at a dose of 25 mg/kg body weight, orally after 2 h of gentamicin injection (80 mg/kg body weight, i.p.) for 8 days and (5) Gentamicin + Nigerloxin (100 mg/kg), treated with nigerloxin at a dose of 100 mg/kg body weight, orally after 2 h of gentamicin injection (80 mg/kg body weight, i.p.) for 8 days. All animals had free access to water and maintained on basal diet (AIN-76) throughout experimental period.

At the end of the experiment, overnight fasted animals were sacrificed over light ether anaesthesia. Blood was collected in tubes by cardiac puncture and serum was separated by centrifugation and stored at −40 °C until further analysis. Kidneys were quickly excised, weighed and processed for further analysis. A 10 % (w/v) kidney homogenate was prepared in 50 mM phosphate buffer (pH 7.4) and centrifuged at 15,000 x g at 4 °C for 30 min. All the biochemical parameters were analyzed in the soluble fraction of kidney homogenate. Another portion kidney was used to measure the lipid peroxides.

Lipid peroxides in kidney

Kidney tissue was homogenized in ice-cold 150 mM KCl (potassium chloride) solution and used for the determination of lipid peroxides. The malondialdehyde content of homogenates was estimated fluorimetrically by measuring the presence of thiobarbituric acid reactive substances (TBARS) according to the method described by Ohkawa et al. (1979). The intensity of emitted fluorescence of the butanol extract was
recorded at 553 nm by excitation at wavelength 515 nm and was compared with the standard tetramethoxy propane.

**Antioxidant molecules**

GSH in kidney was estimated according to the procedure described by Beutler et al. (1963). Total thiols in kidney homogenates were determined spectrometrically as described by Sedlak and Lindsay (1968). Ascorbic acid was measured spectrophotometrically according to the method given by Omaye et al. (1973).

**Antioxidant enzymes in kidney**

Catalase activity was assayed by following the rate of decomposition of hydrogen peroxide as described by Aebi, (1984). Superoxide dismutase activity was measured by quantifying the inhibition of cytochrome-C reduction in xanthine–xanthine oxidase system as described by Flohe and Otting, (1984). Glutathione peroxidase activity was measured as described by Flohe and Gunzler, (1984). Glutathione reductase activity was assayed by measuring the oxidation of NADPH by oxidized glutathione according to Carlberg and Mannervik, (1985). Glutathione-S-transferase activity was assayed using the CDNB and by measuring the CDNB-GSH complex formed as described by Warholm et al. (1985).

**Serum creatinine and urea**

Serum creatinine was measured by the method of Folin and Wu as described by Oser, (1965) by reacting with alkaline picrate solution. The intensity of orange-red colour developed was measured at 520 nm and compared with the standard creatinine. Serum urea was estimated by the method of Levine et al. (1961), by measuring the colour intensity after reacting with \(p\)-dimethylaminobenzaldehyde and expressed as blood urea nitrogen.

**Protein**

Total protein in kidney homogenate was determined by Lowry’s method using BSA as standard (Lowry et al. 1951).
Statistical analysis

All analytical data are expressed as mean ± standard error of mean (SEM). Significance of difference between the groups was evaluated by analysis of variance (ANOVA) followed by Dunnett multiple comparisons test. P<0.05 was considered statistically significant.

Results

Influence of nigerloxin on body weight and kidney weight

The influence of nigerloxin on body weight and kidney weight are presented in Table-1. Body weight and kidney weight of gentamicin treated rats were decreased significantly as compared to control group animals (p<0.05). Nigerloxin administration at dose levels of 25 and 100 mg/kg body weight did not counter this decrease in body and kidney weights. The body and kidney weights were not altered in normal rats treated with nigerloxin compared to untreated control animals.

Influence of nigerloxin on serum urea and creatinine levels

Serum urea and creatinine concentrations of gentamicin-treated rats are presented in Table-2. Gentamicin treatment for 8 days significantly increased the serum creatinine and urea as compared to the control group (p<0.05). Administration of nigerloxin at the higher dose countered this increase in serum creatinine and urea in gentamicin treated rats.

Influence of nigerloxin on kidney lipid peroxides and antioxidant molecules

Beneficial effect of nigerloxin on lipid peroxides measured as TBARS and antioxidant molecules in the kidney of gentamicin treated rats is presented in Table-3. Significant higher concentration of lipid peroxides (p<0.05) was observed in the kidney of gentamicin treated animals as compared to control group of animals. A significant decrease in the kidney TBARS compounds (p<0.05) was seen in gentamycin treated animals with nigerloxin administration at a dose of 100 mg/ kg body weight as compared
**Table-1. Influence of nigerloxin on body weight and kidney weight in gentamicin treated rats**

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Body weight (g)</th>
<th></th>
<th>Kidney weight (g/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Gain in body weight</td>
</tr>
<tr>
<td>Control</td>
<td>173.3 ± 0.71</td>
<td>191.3 ± 1.09</td>
<td>18.0 ± 0.59</td>
</tr>
<tr>
<td>Nigerloxin (100 mg/kg)</td>
<td>172.9 ± 0.59</td>
<td>187.7 ± 1.07</td>
<td>14.8 ± 0.66</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>173.2 ± 0.57</td>
<td>163.8 ± 1.06</td>
<td>−9.4 ± 1.35*</td>
</tr>
<tr>
<td>Gentamicin + Nigerloxin (25 mg/kg)</td>
<td>173.2 ± 0.44</td>
<td>163.3 ± 1.21</td>
<td>−9.9 ± 1.01</td>
</tr>
<tr>
<td>Gentamicin + Nigerloxin (100 mg/kg)</td>
<td>172.8 ± 0.72</td>
<td>165.2 ± 2.11</td>
<td>−7.6 ± 1.52</td>
</tr>
</tbody>
</table>

All values are mean ± SEM of 8 animals in each group.

*: Significantly different from Control group (p<0.05).
Table-2. Influence of nigerloxin on serum creatinine and urea in gentamicin treated rats

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Creatinine (mg/dL)</th>
<th>Urea (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.128 ± 0.020</td>
<td>14.24 ± 0.06</td>
</tr>
<tr>
<td>Nigerloxin (100 mg/kg)</td>
<td>0.215 ± 0.015</td>
<td>14.16 ± 0.06</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>3.961 ± 0.238 *</td>
<td>21.09 ± 1.04 *</td>
</tr>
<tr>
<td>Gentamicin + Nigerloxin (25 mg/kg)</td>
<td>3.448 ± 0.152</td>
<td>19.75 ± 0.23</td>
</tr>
<tr>
<td>Gentamicin + Nigerloxin (100 mg/kg)</td>
<td>3.246 ± 0.250 **</td>
<td>18.40 ± 0.59 **</td>
</tr>
</tbody>
</table>

All values are mean ± SEM of 8 animals in each group.

*: Significantly different from Control group (p<0.05).

**: Significantly different from Gentamicin group (p<0.05).
Table-3. Influence of nigerloxin on lipid peroxides, ascorbic acid, glutathione and total thiols in the kidney of gentamicin treated rats

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Lipid peroxides (nmol/mg protein)</th>
<th>Ascorbic acid (µg/mg protein)</th>
<th>Reduced glutathione (ng/mg protein)</th>
<th>Total thiols (mmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.54 ± 0.183</td>
<td>1.86 ± 0.04</td>
<td>45.82 ± 1.67</td>
<td>0.173 ± 0.009</td>
</tr>
<tr>
<td>Nigerloxin (100 mg/kg)</td>
<td>1.53 ± 0.194</td>
<td>1.90 ± 0.04</td>
<td>45.05 ± 1.31</td>
<td>0.159 ± 0.009</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2.52 ± 0.252*</td>
<td>1.56 ± 0.03*</td>
<td>38.07 ± 1.31*</td>
<td>0.090 ± 0.003*</td>
</tr>
<tr>
<td>Gentamicin + Nigerloxin (25 mg/kg)</td>
<td>2.35 ± 0.185</td>
<td>1.64 ± 0.06</td>
<td>42.63 ± 1.56</td>
<td>0.121 ± 0.003</td>
</tr>
<tr>
<td>Gentamicin + Nigerloxin (100 mg/kg)</td>
<td>1.43 ± 0.205**</td>
<td>1.69 ± 0.04</td>
<td>45.73 ± 1.08**</td>
<td>0.136 ± 0.008**</td>
</tr>
</tbody>
</table>

All values are mean ± SEM of 8 animals in each group.

*: Significantly different from Control group ($p<0.05$).

**: Significantly different from Gentamicin group ($p<0.05$).
to that of untreated gentamycin administered animals. Nigerloxin did not have any influence on lipid peroxides in the kidney of normal animals.

Gentamicin treated animals showed significant decrease in the concentrations of major kidney antioxidant molecules – ascorbic acid, glutathione and total thiols ($p<0.05$) (Table-3). Administration of nigerloxin at a dose of 100 mg/kg body weight significantly elevated glutathione and total thiols in the kidneys of gentamicin treated animals ($p<0.05$). But there was no beneficial influence on levels of ascorbic acid. Nigerloxin at the lower dose of 25 mg/kg body weight did not have any influence on the antioxidant molecules. Nigerloxin did not have any influence on the concentration of antioxidant molecules in the kidney of normal animals.

**Influence of nigerloxin on kidney antioxidant enzymes**

Influence of nigerloxin on the activities of antioxidant enzymes in the kidney of gentamicin administered animals is shown in Table-4. Activities of kidney antioxidant enzymes – superoxide dismutase, catalase, glutathione reductase, glutathione peroxidase and glutathione-S-transferase were significantly decreased in gentamicin treated animals as compared to the control animals ($p<0.05$). Administration of nigerloxin at the dose of 100 mg/kg body weight countered the decrease in activities of each of these enzymes significantly ($p<0.05$) in the kidneys of gentamicin administered animals. Such a beneficial influence was not seen at the lower dose of nigerloxin treatment. These enzyme activities were also not altered by nigerloxin treatment in normal rats.
Table-4. Influence of nigerloxin on kidney antioxidant enzymes in gentamicin treated rats.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Superoxide dismutase(^1) (U/mg protein)</th>
<th>Glutathione-S-transferase(^2) (µmol/min/mg protein)</th>
<th>Glutathione peroxidase(^3) (µmol/min/mg protein)</th>
<th>Glutathione Reductase(^4) (µmol/min/mg protein)</th>
<th>Catalase(^5) (µmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.66 ± 0.107</td>
<td>0.104 ± 0.008</td>
<td>0.110 ± 0.006</td>
<td>0.086 ± 0.006</td>
<td>29.01 ± 1.74</td>
</tr>
<tr>
<td>Nigerloxin (100 mg/kg)</td>
<td>1.60 ± 0.080</td>
<td>0.103 ± 0.004</td>
<td>0.128 ± 0.006</td>
<td>0.088 ± 0.004</td>
<td>31.07 ± 1.23</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1.09 ± 0.068*</td>
<td>0.052 ± 0.001*</td>
<td>0.057 ± 0.002*</td>
<td>0.041 ± 0.001*</td>
<td>17.03 ± 0.81*</td>
</tr>
<tr>
<td>Gentamicin + Nigerloxin (25 mg/kg)</td>
<td>1.20 ± 0.134</td>
<td>0.068 ± 0.006</td>
<td>0.079 ± 0.008</td>
<td>0.057 ± 0.006</td>
<td>23.16 ± 1.34</td>
</tr>
<tr>
<td>Gentamicin + Nigerloxin (100 mg/kg)</td>
<td>1.60 ± 0.047**</td>
<td>0.075 ± 0.002**</td>
<td>0.089 ± 0.005**</td>
<td>0.062 ± 0.003**</td>
<td>24.40 ± 0.79**</td>
</tr>
</tbody>
</table>

All values are mean ± SEM of 8 animals in each group.

*: Significantly different from Control group \((p<0.05)\).

**: Significantly different from Gentamicin group \((p<0.05)\).

1: One unit of activity was taken as the enzyme reaction which inhibits the rate of cytochrome C reduction by 50%.
2: µmol of CDNB-GSH complex formed/min/mg protein.
3,4: µmol of NADPH oxidised/min/mg protein.
5: µmol of H\(_2\)O\(_2\) decomposed/min/mg protein.
Discussion

Results of this study have corroborated with the previous reports in which gentamicin at dose of 80 mg/kg/body weight significantly produced nephrotoxicity (Silan et al. 2007; Soliman et al. 2007). Gentamicin distinctively accumulates in epithelial cells of the proximal tubules of the kidneys and alters the functions of several organelles and processes that are crucial for cell viability (Quiros et al. 2011). It is well evidenced free radicals play a key role in gentamicin induced nephrotoxicity in rats. It has been reported that gentamicin directly increases the production of mitochondrial ROS from the respiratory chain. ROS cause cellular damage and death through diverse mechanisms (Morgan et al. 2007; Ott et al. 2007; Ryter et al. 2007). ROS have been proposed as a causative agent of cell death in many different pathological states as well as, in glomerular disease (Smetana et al. 1988), in renal ischemia and reperfusion injury (Longoni et al. 2002), and in models of toxic renal failure (Piotrowski et al. 1996).

Many studies have shown an elevation in the concentration of biochemical marker of kidney function such as serum urea and creatinine in gentamicin treated rats. We observed in our study that serum urea and creatinine were increased after gentamicin treatment indicating glomerular damage. The co-administration of nigerloxin with gentamicin to rats resulted in a significant reduction in the elevated levels of serum urea and creatinine. Several other researchers also reported antioxidants such as resveratrol and carnosine prevented the increase in serum urea and creatinine levels induced by gentamicin (Silan et al. 2007; Soliman et al. 2007).

Nephrotoxicity of gentamicin has been associated with an increase in lipid peroxide accumulation in the kidney tissue. Gentamicin causes rapid changes in membrane lipid composition which leads to free radicals initiated lipid peroxidation. Increased hydroxyl radical has been postulated to induce renal tissue damage by causing peroxidation of membrane lipids. In the present study, gentamicin treated rats exhibited an increase in lipid peroxide levels in the kidney. Other researchers also have reported increased MDA levels, one of the products of lipid peroxidation, in gentamicin treated rats (Sener et al.
Co-administration of nigerloxin with gentamicin significantly reduced lipid peroxide levels accumulation in the kidney of experimental animals. ROS generated during normal cellular functions are scavenged by intrinsic antioxidant defense systems. Enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase and major antioxidant molecule GSH play a major role in removing the free radicals. Increase in kidney reactive oxygen species level by gentamicin (e.g. $\cdot O_2^-$, H$_2$O$_2$, and peroxynitrite) could induce the inactivation of manganese-superoxide dismutase, glutathione peroxidase, glutathione reductase, and catalase in renal cortex of gentamicin-treated rats. It is known that peroxynitrite anions impair manganese-superoxide dismutase (MacMillan-Crow and Thompson, 1999) and glutathione peroxidase activity (Padmaja et al. 1998) and $\cdot O_2^-$ inactivates glutathione peroxidase and catalase (Rister and Baehner, 1976). In our study, activities of antioxidant enzymes were significantly reduced in the kidneys of gentamicin treated rats indicating the dominance of oxidative stress. Other researchers have also reported reduction in antioxidant enzyme activities in the kidney of gentamicin treated rats (Pedraza-Chaverri et al. 2004). Nigerloxin significantly prevented the decrease in superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase activities and thus improve the antioxidant status of rat kidney.

Glutathione has a very important role in protecting against oxygen free radical damage by providing reducing equivalents for several enzymes. GSH is also a scavenger of hydroxyl radicals and singlet oxygen (Masella et al. 2005). Further, GSH contributes to antioxidant defense by reducing oxidized ascorbic acid (dehydroascorbate) back to its active form. In the present study, the levels of total thiols and GSH in rat kidney tissues were significantly reduced after gentamicin injection compared with control group. Reduction in kidney GSH levels after gentamicin administration has also been reported by other researchers (Silan et al. 2007; Soliman et al. 2007). Nigerloxin possesses antioxidant potential of countering the decrease in levels of total thiols and GSH in the kidney of gentamicin treated rats.
Thus, we observed that gentamicin nephrotoxicity is associated with enhanced blood urea and creatinine levels, lipid peroxide accumulation in kidney and compromised antioxidant defense system in kidney. Oral administration of nigerloxin, a fungal metabolite countered lipid peroxidation in the kidneys of gentamicin treated rats. Serum urea and creatinine levels were effectively reduced by nigerloxin administration. The results also suggest the beneficial influence of nigerloxin in potentiating the antioxidant defense system in this experimental model. Elevated activities of antioxidant enzymes and concentrations of antioxidant molecules in the nigerloxin administered animals indicate that this fungal metabolite has the potential of reducing the oxidative stress induced by gentamicin.

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Summary

Oxidative stress plays a key role in gentamicin induced nephrotoxicity in rats. We investigated antioxidant potential of a novel fungal metabolite, nigerloxin, a potent aldose reductase inhibitor and a free radical scavenger in this experimental model. Experimental kidney toxicity was induced in male Wistar rats by administration of gentamicin at a dose of 80 mg/kg, i.p., for a period of 8 days. Groups of rats were orally administered with nigerloxin for 8 days at a dose of 25 mg and 100 mg/kg body weight/day along with gentamicin. Gentamicin induced increase in lipid peroxides, decrease in glutathione, total thiols and activities of antioxidant enzymes — catalase, glutathione peroxidase, super oxide dismutase, glutathione reductase and glutathione-S-transferase in the kidney. Blood creatinine and urea concentrations were significantly increased. Blood creatinine and urea levels were reduced by nigerloxin treatment. Nigerloxin treated rats also exhibited elevated activities of superoxide dismutase, glutathione peroxidase, glutathione reductase, catalase and glutathione-S-transferase in kidney. Glutathione and total thiols in kidney were elevated in nigerloxin treated rats. Thus, nigerloxin ameliorated oxidative stress induced by gentamicin in the renal tissue.

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