CHAPTER - 3

BENEFICIAL INFLUENCE OF NIGERLOXIN ON EYE LENS ABNORMALITIES
Section - A

BENEFICIAL INFLUENCE OF NIGERLOXIN ON EYE LENS ABNORMALITIES IN EXPERIMENTAL DIABETES

Introduction

Cataract accounts for a major cause of blindness worldwide. Cataract is the most common visual impairment in both type 1 and type 2 diabetic patients. The pathogenesis of diabetic cataract is still not fully understood. The hyperosmotic and oxidative stress theories are well studied and considered to be the major biochemical mechanisms in the development of diabetic cataract (Brownlee, 2001). Aldose reductase (ALR), a key enzyme in the polyol pathway identifies as the primary factor responsible for the pathological alterations by osmotic stress in diabetic cataract. The enzyme ALR catalyses the conversion of glucose to sorbitol, cause an intermolecular osmotic imbalance which promotes the occurrence of a cascade of events leading to lens opacification (Kinoshita et al. 1979). In addition, oxidative stress in the eye lens also plays a major role in the initiation and progression of various types of cataracts, including diabetic cataract. Oxidative stress is increased in diabetes because of multiple factors. Glucose autoxidation is the major source of free radicals in diabetes (Wolff and Dean, 1987). The nonenzymatic glycation of proteins (Mullarkey et al. 1990), and interaction between glycated products and their receptors (Schmidt et al. 1994), and also polyol pathway (Cheng and Gonzalez, 1986; Brownlee, 2001) are potential source of hyperglycemia induced oxidative stress. Other factors include cellular oxidation/reduction imbalances, reduction in antioxidant defenses – both antioxidant molecules and antioxidant enzymes that dispose of free radicals.

Aldose reductase inhibitors (ARIs) and some antioxidants have added beneficial influence in diabetic cataract and its complication but none have achieved clinical success for diverse reasons. The beneficial influence of ARIs has been tested in various animal models as potential agents to prevent diabetic cataracts and to ameliorate other
complications of diabetes (Chylack et al. 1979; Stribling et al. 1985; Griffin et al. 1987; Kato et al. 1991; Robinson et al. 1996; Oates and Mylari, 1999). A new ARI, nigerloxin (2-amido-3-hydroxy-6-methoxy-5-methyl-4-(prop-1'-enyl) benzoic acid obtained from solid state fermentation of Aspergillus niger (CFR-W-105) has been evidenced to exhibit inhibitory activity on the partially purified rat lens ALR (Rao et al. 2002; Rao et al. 2005) and free radical scavenging activity in vitro (Chapter 2A). Our animal study (Chapter 2B) has indicated the beneficial influence of nigerloxin on oxidative stress associated with diabetes which may have an implication in delaying or ameliorating the secondary complications. The present animal study was carried out to evaluate the ALR inhibitory potential of nigeloxin in vivo and the beneficial influence of nigerloxin if any on the modulation of eye lens injuries in streptozotocin induced diabetes in experimental rats.

Materials and methods

Chemicals

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

Nigerloxin production

Nigerloxin was obtained through solid state fermentation of Aspergillus niger CFR-W-105 (Rao et al. 2002; Rao et al. 2005) as described 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).
**Induction of diabetes**

The animal study was carried out taking appropriate measures to minimize pain or discomfort and with due approval from the Institutional Animal Ethics Committee. Male Wistar rats of 10 weeks age and body weight 140–150 g raised by Experimental Animal Production Facility Unit of this Institute were used in this investigation. Experimental diabetes was induced by a single intra peritoneal (i.p.) injection of streptozotocin to animals fasted overnight at a dose of 50 mg/kg body weight as 1 mL in freshly prepared 0.1M citrate buffer (pH 4.5) and the control rats received 0.1M citrate buffer (pH 4.5) alone. One week after the injection of streptozotocin, blood samples were obtained from retro orbital plexus from overnight fasted animals. Fasting blood glucose levels were determined by glucose oxidase method. Rats with fasting blood glucose levels above 300 mg/dL were sorted and used as diabetic animals.

**Animal treatment**

Experimental animals (8 per group) were housed individually in stainless steel cages under standard laboratory conditions at room temperature with 12 h light and dark cycles. Three groups of diabetic animals and a parallel 2 groups of normal animals were maintained on basal diet (AIN-76) *ad libitum* with free access to water. One group of diabetic animals received nigerloxin at a dose of 25 mg/kg body weight and another group of diabetic animals received nigerloxin 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 (non-diabetic) 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 the vehicle (3% aqueous starch) alone. At the end of 30 days, overnight fasted animals were sacrificed over 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.

Lens separated from both the eyes were processed for further analysis. A 10 % (w/v) lens 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 lens homogenate except for lens malondialdehyde (MDA), which was determined in the total homogenate of lens.

**Lens polyol pathway enzymes**

Activity of lens aldose reductase was measured spectrophotometrically (Shimadzu UV-1800; Shimadzu Corporation, Kyoto, Japan) according to the method of Kim and Oh, (1999). Briefly, the assay mixture contained sodium-potassium-phosphate buffer (0.135M, pH 7.0), 0.32 mM NADPH, and lens sample. 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 Hibi, (1974). Briefly, the assay mixture contained triethanolamine buffer (0.2M, pH 7.4), 0.0012 mM NADH, and enzyme solution. The reaction was initiated by the addition of 1.19 mM fructose solution and monitored by following the decrease in absorbance at 365 nm for 5 min.

**Lens 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 315–400 nm.

**Lipid peroxides in lens**

Lens lipid peroxides were assayed by measuring the malondialdehyde (MDA) concentration as thiobarbituric acid reactive substances (TBARS) fluorometrically 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 tetraethoxy propane.
**Antioxidant molecules in lens**

Reduced glutathione was estimated according to the protocol described by Beutler et al. (1963). Total thiols in lens homogenates were determined spectrometrically as described by Sedlak and Lindsay, (1968).

**Antioxidant enzymes in lens**

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-S-transferase activity was assayed using the chlorodinitrobenzene and by measuring the CDNB-GSH complex formed as described by Warholm et al. (1985).

**Glucose and protein**

Plasma glucose level was determined by glucose oxidase peroxidase method of Huggett and Nixon, (1957). Total protein in soluble fraction of lens homogenate was determined by Lowry’s method using BSA as standard (Lowry et al. 1951).

**Opthalmological examination**

Eye lenses of the experimental animals were examined for cataract associated changes at the end of the experimental period using a slit lamp equipped with a built-in camera (Carl Zeiss Meditech AG, 07740 Jena, Germany).

**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 lens polyol pathway enzymes

The influence of nigerloxin on the activities of polyol pathway enzymes in rat eye lens is presented in Table-1. Lens aldose reductase and sorbitol dehydrogenase activities were significantly \( (p<0.05) \) increased in diabetic rats as compared to normal animals. The elevation in the activity of lens aldolase reductase was 37\%, while sorbitol dehydrogenase was increased by 133\%. Nigerloxin administration at 100 mg/kg body weight significantly \( (p<0.05) \) countered the increase in both these enzyme activities in the lens of diabetic animals (by 22 and 34\%, respectively). The lower dose, 25 mg/kg body weight of nigerloxin did not produce any appreciable decrease in the same. The enzymes activities were not altered in normal rats treated with nigerloxin compared to untreated normal rats.

Influence of nigerloxin on lens AGEs and tryptophan fluorescence

Lens AGEs and tryptophan related fluorescence intensity data are represented in Fig.1 and Fig.2 respectively. AGEs related fluorescence of lens soluble protein was significantly increased in diabetic animals (Fig.1). Oral administration of nigerloxin at the higher dose countered the formation of AGEs in diabetic lens (Fig.1). On the other hand, tryptophan related fluorescence decreased markedly in diabetic rat lens (Fig.2). Nigerloxin administration especially at the higher dose countered this alteration of protein in the lens of diabetic animals.

Influence of nigerloxin on lens lipid peroxides and antioxidant molecules

Beneficial effect of nigerloxin on lipid peroxidation measured as TBARS in the lens of diabetic rats is presented in Table-2. Significantly high concentration of lipid peroxides \( (p<0.05) \) was observed in the lens of diabetic rats compared to that of normal control animals. A significant decrease in the lens TBARS compounds \( (p<0.05) \) was seen in diabetic animals treated with nigerloxin at a dose of 100 mg/kg body weight as compared to that of untreated diabetic animals. Nigerloxin did not have any influence on
Table-1. Influence of nigerloxin on the activities of lens aldose reductase and sorbitol dehydrogenase in diabetic rats.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Aldose Reductase&lt;sup&gt;1&lt;/sup&gt; (µmol/h/100 mg protein)</th>
<th>Sorbitol Dehydrogenase&lt;sup&gt;2&lt;/sup&gt; (µmol/h/100 mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>17.5 ± 0.83</td>
<td>0.78 ± 0.132</td>
</tr>
<tr>
<td>Nigerloxin (100 mg/kg)</td>
<td>17.4 ± 1.10</td>
<td>0.76 ± 0.037</td>
</tr>
<tr>
<td>Diabetic</td>
<td>23.9 ± 0.83*</td>
<td>1.82 ± 0.086*</td>
</tr>
<tr>
<td>Diabetic + Nigerloxin (25 mg/kg)</td>
<td>23.1 ± 1.64</td>
<td>1.65 ± 0.180</td>
</tr>
<tr>
<td>Diabetic + Nigerloxin (100 mg/kg)</td>
<td>18.6 ± 0.25**</td>
<td>1.20 ± 0.075**</td>
</tr>
</tbody>
</table>

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

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

**: Significantly ($P<0.05$) different from Diabetic group.

1: µmol NADPH oxidized/h/100 mg protein; 2: µmol NADH oxidized/h/100 mg protein.
Fig. 1A. Influence of nigerloxin on AGE’s fluorescence of soluble protein in diabetic rat lens. Protein (1 mg/mL) in 0.05 M sodium phosphate buffer pH 7.4 was excited at 370 nm and emission was monitored 400-500 nm. Data represent mean of 8 animals in each group.

Fig. 1B. Influence of nigerloxin on AGEs related fluorescence of soluble protein in diabetic rat lens. Data represent fluorescence intensity at 422 nm. All values are mean ± SEM of 8 animals in each group; a, significantly ($P<0.01$) different from Control group; b, significantly ($P<0.01$) different from Diabetic group.
Fig. 2A. Influence of nigerloxin on tryptophan fluorescence of soluble protein in diabetic rat lens. Protein (1 mg/mL) in 0.05 M sodium phosphate buffer pH 7.4 was excited at 295 nm and emission was monitored 300-400 nm. Data represent mean of 8 animals in each group.

Fig. 2B. Influence of nigerloxin on tryptophan fluorescence of soluble protein in diabetic rat lens. Data represent fluorescence intensity at 332 nm. All values are mean ± SEM of 8 animals in each group; a, significantly (P<0.01) different from Control group; b, significantly (P<0.01) different from Diabetic group.
lipid peroxides in the lens of normal animals. The influence of nigerloxin on the concentration of lens antioxidant molecules is presented in Table-2. Diabetic animals showed significant decrease in the concentrations of lens antioxidant molecules – glutathione and total thiols. Administration of nigerloxin at a dose of 100 mg/kg body weight significantly (p<0.05) elevated these antioxidant molecules in the lens of diabetic animals. Nigerloxin at the lower dose (25 mg/kg body weight) did not have any influence on the same.

**Influence of nigerloxin on lens antioxidant enzymes**

The influence of nigerloxin on lens antioxidant enzymes of diabetic animals is shown in Table-3. Activities of lens antioxidant enzymes – superoxide dismutase, glutathione peroxidase and glutathione-S-transferase were significantly decreased in diabetes as compared to the normal control animals, the decreases in these enzyme activities being 32%, 27%, and 21%, respectively. Administration of nigerloxin at a dose of 100 mg/kg body weight countered the decrease in each of these enzyme activities significantly (p<0.05) in the eye lens of diabetic animals. Such a beneficial influence was not seen at the lower dose of nigerloxin treatment. The enzyme activities in normal rats were not altered by nigerloxin treatment.

**Protective effect of nigerloxin administration on cataractogenesis in diabetic rats**

Influence of nigerloxin on the cataractogenesis and lenticular opacity of the eye lens in diabetic rats is evident in Fig.3. Slit lamp examination of the rat eyes was conducted to observe the cataract associated changes in the eye lens. The incidence of mature cataract was considerably lower in diabetic animals administered nigerloxin at the higher dose, with only one out of eight of the diabetic control group of animals having developed mature cataract compared to the diabetic control group of animals (seven out of eight of the diabetic control group exhibited mature cataract) at the end of the experimental period. The observations clearly revealed the presence of very mild lenticular opacity and posterior subcapsular cataract (immature cataract) in the diabetic group of animals administered nigerloxin in contrast to the significant lenticular opacity (mature cataract)
**Table-2.** Influence of nigerloxin on lens lipid peroxides and glutathione in diabetic rats

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Lipid peroxides (nmol/mg protein)</th>
<th>Reduced glutathione (µg/mg protein)</th>
<th>Total thiols (mM/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.484 ± 0.017</td>
<td>103.7 ± 3.68</td>
<td>2.281 ± 0.071</td>
</tr>
<tr>
<td>Nigerloxin (100 mg/kg)</td>
<td>0.528 ± 0.060</td>
<td>98.0 ± 4.81</td>
<td>2.260 ± 0.060</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.952 ± 0.028*</td>
<td>48.4 ± 1.94*</td>
<td>0.916 ± 0.042*</td>
</tr>
<tr>
<td>Diabetic+ Nigerloxin (25 mg/kg)</td>
<td>1.093 ± 0.090</td>
<td>56.4 ± 6.83</td>
<td>0.933 ± 0.022</td>
</tr>
<tr>
<td>Diabetic+ Nigerloxin (100 mg/kg)</td>
<td>0.645 ± 0.053**</td>
<td>69.2 ± 3.72**</td>
<td>1.294 ± 0.043**</td>
</tr>
</tbody>
</table>

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

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

**: Significantly (P<0.05) different from Diabetic group.
Table-3. Influence of nigerloxin on lens antioxidant enzymes in diabetic rats.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Superoxide dismutase(^1) (U/mg protein)</th>
<th>Glutathione-S-transferase(^2) (nmol/min/mg protein)</th>
<th>Glutathione peroxidase(^3) (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.892 ± 0.119</td>
<td>2.270 ± 0.077</td>
<td>1.583 ± 0.063</td>
</tr>
<tr>
<td>Nigerloxin (100 mg/kg)</td>
<td>1.697 ± 0.075</td>
<td>1.964 ± 0.053</td>
<td>1.570 ± 0.061</td>
</tr>
<tr>
<td>Diabetic</td>
<td>1.287 ± 0.091 *</td>
<td>1.655 ± 0.042 *</td>
<td>1.247 ± 0.031 *</td>
</tr>
<tr>
<td>Diabetic + Nigerloxin (25 mg/kg)</td>
<td>1.400 ± 0.033</td>
<td>1.744 ± 0.079</td>
<td>1.442 ± 0.046</td>
</tr>
<tr>
<td>Diabetic + Nigerloxin (100 mg/kg)</td>
<td>1.776 ± 0.042 **</td>
<td>2.066 ± 0.065 **</td>
<td>1.699 ± 0.117 **</td>
</tr>
</tbody>
</table>

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

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

**: Significantly \((P<0.05)\) different from Diabetic group.

1: One unit of activity was taken as the enzyme reaction which inhibits the rate of cytochrome C reduction by 50%.

2: \(\mu\text{mol of CDNB-GSH complex formed/min/mg protein}\).

3: \(\mu\text{mol of NADPH oxidised/min/mg protein}\).
Fig. 3. Influence of nigerloxin on the cataractogenesis and lenticular opacity of the eye lens in diabetic rats. (A) Normal eyes and absence of lenticular opacity in the normal control group, (B) Corneal vascularisation, significant lenticular opacity, and mature cataract found in rats of diabetic control group, (C) Immature posterior subcapsular cataract found in nigerloxin (25 mg/kg) administered diabetic rats, and (D) Almost countering of the cataractogenesis in nigerloxin (100 mg/kg) administered diabetic rats.
and corneal vacuolization observed in the diabetic control group of animals (Fig.3). Nigerloxin administration at the lower dose of 25 mg/kg to diabetic rats had only a partial beneficial effect, where immature posterior subcapsular cataract could still be found. No lenticular opacity was observed in the non-diabetic animals. These observations indicated that nigerloxin delays cataractogenesis in diabetic animals.

**Discussion**

The central role of polyol pathway induced oxidative stress has been extensively studied in the diabetic cataract formation (Chung et al. 2003). Osmotic hypothesis of sugar cataract formation, emphasizing that the intracellular increase of fluid in response to ALR-mediated accumulation of sorbitol results in lens swelling associated with complex biochemical changes ultimately leading to cataract formation (Kinoshita, 1974; Kinoshita et al. 1979; Kador and Kinoshita, 1984). Apoptosis of epithelial cells of lens due to sorbitol accumulation has also been reported (Takamura et al. 2001). Accumulation of sorbitol in the endoplasmic reticulum leads to the free radical generation which causes oxidative stress and results in damage to lens fibers. In the present investigation, aldose reductase and sorbitol dehydrogenase activities were significantly increased in diabetic eye lens. Nigerloxin treatment significantly inhibited this elevation in aldose reductase and sorbitol dehydrogenase activities in diabetic animals.

We observed opacification of lens in untreated diabetic rats 5 weeks after STZ administration. Although there are no previous reports on opacification of lenses in such duration in induced diabetes, the same has been observed as early as 24 days in dietary galactose-induced cataractogenesis (Suryanarayan et al. 2003).

Hyperglycemia induced glycation of lens proteins leads to the formation of AGEs as one of the major source for the generation of superoxide radicals (\( \cdot O_2^- \)) (Stitt, 2005). 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). Recently, the contribution of
osmotic and oxidative stress in cataract development has been shown in sorbitol dehydrogenase deficient mice (Chan et al, 2008). In our study, we also noticed increased accumulation of AGEs in the lens of diabetic rats by measuring AGEs related fluorescence. Nigerloxin treatment significantly inhibited the formation of AGEs in the eye lens of diabetic animals.

In the present study, increased lipid peroxides along with the decreased concentration of glutathione and compromised activities of antioxidant enzymes in rat lens suggest increased oxidative stress in diabetic condition. The increased level of TBARS suggests increased levels of free radicals which could be due to their increased production or decreased destruction in diabetic status. Nigerloxin treatment significantly reduced this accumulation of lipid peroxides.

Glutathione, a major non-enzymatic antioxidant provides the first line of defense against reactive oxygen radicals. Glutathione plays a major protective role against oxidative stress (Masella et al. 2005) by scavenging hydroxyl radical and singlet oxygen directly, detoxifying hydrogen peroxide and lipid peroxides by the catalytic action of glutathione peroxidase. Further glutathione contributes to antioxidant defense by reducing oxidized vitamin C (dehydroascorbate) to its active form. The decreased concentration of glutathione in lens may be due to NADPH depletion or glutathione consumption in the removal of peroxides. Nigerloxin treatment to diabetic rats led to a recovery in the concentration of lens glutathione. This effect may be due inhibitory potential of nigerloxin on aldose reductase enzyme resulting in decreased usage of NADPH. It has been reported that other aldose reductase inhibitors, such sorbinil, at the precataractous stage of diabetes could correct the biochemical abnormalities in the lens (Obrosova and Fathallah, 2000). They observed that sorbinil treatment essentially corrected diabetes-induced sorbitol and fructose accumulation. MDA accumulation, reduced glutathione depletion and the increase in oxidized glutathione: reduced glutathione ratio was partially corrected.
Major antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and glutathione-S-transferase activities were decreased in the eye lens of diabetic animals indicating the dominance of oxidative stress in diabetic eye lens. Superoxide dismutase scavenges superoxide anion radicals and reduces the deleterious effects of superoxide anion radicals. The decreased superoxide dismutase activity may be due to glycation and inactivation of enzyme in eye lens of diabetic rats (Ookawara et al. 1992). Glutathione peroxidase serves to detoxify peroxides by using reduced glutathione as a substrate. Low glutathione peroxidase activity in diabetic lens could be due to decreased concentration of the substrate glutathione. Glutathione-S-transferase catalyses the reductions of hydrogen peroxide and hydro peroxides to non-toxic products. Reduction in the activities of these enzymes augments the deleterious influence of oxidative stress during the development of diabetic cataract. In this context, increased activities of the components of this antioxidant defense system could be important in the protection against radical damage. Nigerloxin possesses antioxidant potential by elevating the activities of all these three antioxidant enzymes in eye lens.

In conclusion, we observed that uncontrolled diabetes in rats is associated with enhanced activity of polyol pathway enzymes and compromised antioxidant defense system. Our experimental data suggest that nigerloxin countered lipid peroxidation and advanced glycation end product formation in the lens of diabetic rats. The results here suggest the beneficial influence of nigerloxin on polyol pathway enzymes and potentiation of antioxidant defense system in experimental diabetic animals. Lowered levels of advanced glycation end products, aldose reductase activity and lesser lenticular opacity as well as the absence of mature cataract in the nigerloxin administered diabetic animals indicate that the this fungal metabolite (a potential aldose reductase inhibitor) prevents protein glycation and delays cataractogenesis in diabetic animals. This is the first report on the potential of this fungal metabolite in the management of diabetic complications, particularly to delay as well as ameliorate hyperglycemia induced cataractogenesis in an animal model.
Summary

Role of osmotic and oxidative stress has been implicated in the pathogenesis of diabetic cataract. Nigerloxin, a fungal metabolite has been shown to possess aldose reductase inhibitory and free radical scavenging potential in vitro. In the present study, the beneficial influence of nigerloxin was investigated on diabetes induced alteration in the eye lens of streptozotocin administered rats. Groups of diabetic rats were orally administered nigerloxin (100 mg/kg/day) for 30 days. Activities of lens polyol pathway enzymes – aldose reductase and sorbitol dehydrogenase, lipid peroxides and advanced glycation end products (AGEs) were increased in diabetic animals. Glutathione and activities of antioxidant enzymes – superoxide dismutase, glutathione-S-transferase and glutathione peroxidase were decreased in the eye lens of diabetic animals. Administration of nigerloxin significantly decreased lens lipid peroxides and AGEs in diabetic rats. Increase in lens aldose reductase and sorbitol dehydrogenase activities was countered by nigerloxin treatment. Lens glutathione and antioxidant enzyme activities were significantly elevated in nigerloxin treated diabetic rats. Examination of rat eyes indicated that nigerloxin delayed cataractogenesis in diabetic rats. The results suggest the beneficial countering of polyol pathway enzymes and potentiation of antioxidant defense system by nigerloxin in diabetic animals, implicating its potential in ameliorating diabetic cataract.
Section - B

BENEFICIAL INFLUENCE OF NIGERLOXIN ON EYE LENS ABNORMALITIES IN GALACTOSE FED RATS

Introduction

Activation of polyol pathway is well accepted as the key event in diabetes induced cataractogenesis. Aldose reductase (ALR) is the first enzyme of this pathway which has been implicated as the common factor which initiates the cataractogenic process in both diabetic and juvenile situation. The rate-limiting step of the polyol pathway is the reduction of glucose to sorbitol catalyzed by aldose reductase. Sorbitol is subsequently converted to fructose by the second enzyme sorbitol dehydrogenase. In hyper-galactosemic condition, ALR converts galactose to polyol, galactitol. These polyols are less able to leave the cell, and their concentration builds up inside the cells. Elevated levels of polyols are believed to prompt swelling and disruption of the normal cellular architecture via exerting osmotic stress leads to development of cataract (Kinoshita, 1965; Kinoshita, 1974). On the other hand, active polyol pathway is also associated with oxidative stress (Ansari et al. 1996; Patrick et al. 1985; Srivastava et al. 1989). The over utilization of nicotinamide adenine dinucleotide phosphate reduced (NADPH) by aldose reductase, markedly decreases the reduction of oxygen species via the glutathione reductase/glutathione peroxidase system may contribute to cellular injury. A galactose-rich diet induces a severe degree of cataract than does diabetes in experimental animals since aldose reductase has a greater affinity towards galactose than glucose. It is one of the common animal model used to assess the anticataract potential of chemical moieties. Polyol pathway and oxidative stress are closely associated with induction of cataract. Therefore an aldose reductase inhibitor (ARI) with antioxidant potential may contribute to the amelioration of cataract.

Nigerloxin [2-amido-3-hydroxy-6-methoxy-5-methyle-4-(prop-1’-enyl) benzoic acid] is a fungal metabolite obtained from solid-state fermentation of Aspergillus niger
Nigerloxin, is an aldose reductase (IC$_{50}$ = 69 µM) and lipoxygenase inhibitor, and is also a free radical scavenger in vitro (Chapter-2A). ED$_{50}$ = 68 µM against 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radicals (Rao et al. 2002). The results of our earlier study suggested the beneficial countering of polyol pathway enzymes and potentiation of antioxidant defense system in the eye lens by nigerloxin in diabetic animals, implicating its potential in ameliorating diabetic cataract. In the present study we have examined the influence of nigerloxin on dietary galactose induced juvenile cataract in rats.

**Materials and methods**

**Chemicals**

Nigerloxin was obtained through solid state fermentation of *Aspergillus niger* (CFR-W-105 in house, CFTRI), Mysore, India (Rao et al. 2005) as described in the earlier chapter sections. Galactose was purchased from Sisco Research Laboratories (Mumbai, India). Nicotinamide adenine dinucleotide phosphate reduced (NADPH), nicotinamide adenine dinucleotide (reduced form) (NADH), adenosine-5’-diphosphate (ADP), thiobarbituric acid, glyceraldehyde, reduced glutathione (GSH), glutathione reductase, cytochrome-C, xanthine, xanthine oxidase, tert-butyl hydroperoxides (TBHP), 1-chloro-2,4-dinitrobenzene (CDNB), 5,5’-dithiobis-2-nitrobenzoate (DTNB), ethylene diamine tetra acetic acid (EDTA), fructose, tetraethoxy propane (TEP) and bovine serum albumin (BSA) were obtained from Sigma Aldrich Co. (St. Louis, MO, USA). All other chemicals and solvents used were of analytical grade obtained from Sisco Research Laboratories (Mumbai, India).

**Induction of cataract**

The animal study was carried out with due approval from the Institutional Animal Ethics Committee. Male Wistar rats having an average body weight of 50-55 g raised by the Experimental Animal Production Facility Unit of this Institute were randomized into five groups (n=8 per group). Experimental cataract was induced by maintaining on 30% galactose containing diet. Two groups of animals were maintained on basal diet (AIN-76)
and the other three groups of animals received galactose-rich diet. One group of galactose fed animals received nigerloxin at a dose of 25 mg/kg body weight and another group of galactose-fed animals received nigerloxin at a dose of 100 mg/kg body weight as 0.5 mL solution in 3% aqueous starch orally once a day for 24 days. One group of basal diet fed animals also received nigerloxin at a dose of 100 mg/kg body weight orally once a day for 24 days. The animals were housed in stainless steel cages under standard laboratory conditions at room temperature with 12 h light and dark cycles. All the animals had free access to water and their respective diets.

At the end of the experiment, overnight fasted animals were sacrificed over light ether anaesthesia. Eye lens was separated by the posterior approach from both the eyes and were processed for further analysis. A 10 % (w/v) lens homogenate was prepared from 2 pooled lenses 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 lens homogenate except for lens malondialdehyde (MDA), which was determined in the total homogenate of lens.

**Lens polyol pathway enzymes**

Activity of ALR enzyme in the eye lens was measured spectro-photometrically (Shimadzu UV-1800) according to the method of Kim and Oh, (1999). Briefly, the assay mixture contained sodium-potassium phosphate buffer (0.135 M, pH 7.0), 0.32 mM NADPH, and enzyme solution. 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). Briefly, the assay mixture contained triethanolamine buffer (0.2 M, pH 7.4), 1.2 µM NADH, and enzyme solution. The reaction was initiated by the addition of 1.19 mM fructose solution and monitored by following the decrease in absorbance at 365 nm for 5 min.
Advanced glycation end products related and tryptophan fluorescence in lens

Advanced glycation end products (AGEs) and tryptophan fluorescence was measured in soluble protein of lens using 50 mM sodium phosphate buffer, pH 7.4. AGEs fluorescence spectra were obtained from 400-500 nm with excitation at 370 nm (Monnier and Cerami, 1981) and tryptophan fluorescence spectra were obtained at excitation 295 nm and emission between 315–400 nm in a spectrofluorometer (Shimadzu RF-50301PC).

Lipid peroxides and antioxidant molecules in lens

Malondialdehyde (MDA) concentration in lens homogenate was determined as thiobarbituric acid reactive substances (TBARS) fluorimetrically (Shimadzu RF-50301PC) 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 tetraethoxy propane. The concentration of reduced glutathione in lens was determined according to the protocol described by Beutler et al. (1963). Total thiols in lens were estimated spectrophotometrically as described by Sedlak and Lindsay, (1968).

Antioxidant enzymes in lens

The activity of superoxide dismutase 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-S-transferase activity was assayed by monitoring formation of conjugate, CDNB-GSH between CDNB and GSH as described by Warholm et al. (1985). The protein content of soluble and total homogenate of lens was estimated by employing the method of Lowry et al. (1951), using BSA as the standard.

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 weights

Administration of nigerloxin at 25 and 100 mg/kg body weight did not have any significant effect on body weights of the animals fed galactose-rich diet compared to galactose fed control animals. Body weights of animals maintained on basal diet also were not altered by nigerloxin treatment as compared to untreated animals (Data not shown).

Influence of nigerloxin on lens polyol pathway enzymes

The influence of nigerloxin on the specific activities of eye lens aldose reductase and sorbitol dehydrogenase enzymes is presented in Table-1. Lens aldose reductase enzyme activity was significantly ($p<0.05$) increased in galactose fed animals as compared to normal animals. Nigerloxin administration at 100 mg/kg body weight significantly ($p<0.05$) countered this increase in enzyme activity in the lens of galactose fed animals. The lower dose of nigerloxin, 25 mg/kg body did not produce any favorable decrease in the same. On the other hand, sorbitol dehydrogenase activity was unaltered by galactose feeding or nigerloxin administration in experimental animals. The enzyme activity was also not altered in normal rats treated with nigerloxin compared to untreated normal rats.

Influence of nigerloxin on lens AGEs and tryptophan fluorescence

Beneficial influence of nigerloxin on lens AGEs and tryptophan related fluorescence intensity are presented in Fig.1 and Fig.2 respectively. AGEs levels were significantly increased ($p<0.01$) as indicated by increased fluorescence intensity of lens soluble protein in galactose fed animals as compared to normal control animals. Nigerloxin administration significantly countered the formation of AGEs in the lens of galactose fed rats. On the other hand, tryptophan related fluorescence decreased markedly ($p<0.01$) in the lens of galactose fed rats. Oral administration of nigerloxin at the higher dose countered this alteration in the lens of galactose fed rats.
Table-1. Influence of nigerloxin on the activities of lens aldose reductase and sorbitol dehydrogenase in galactose fed rats.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Aldose Reductase(^1) (µmol/h/100 mg protein)</th>
<th>Sorbitol Dehydrogenase(^2) (µmol/h/100 mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose Control</td>
<td>23.5 ± 1.58(^a)</td>
<td>6.28 ± 0.24</td>
</tr>
<tr>
<td>Galactose + Nigerloxin (25 mg/kg)</td>
<td>21.8 ± 0.80</td>
<td>6.09 ± 0.24</td>
</tr>
<tr>
<td>Galactose + Nigerloxin (100 mg/kg)</td>
<td>17.8 ± 0.75(^b)</td>
<td>5.94 ± 0.08</td>
</tr>
<tr>
<td>Normal Control</td>
<td>11.8 ± 0.97</td>
<td>5.89 ± 0.17</td>
</tr>
<tr>
<td>Normal + Nigerloxin (100 mg/kg)</td>
<td>11.4 ± 0.55</td>
<td>5.95 ± 0.15</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 Galactose Control group.

1: µmol NADPH oxidized/h/100 milligram of protein.

2: µmol NADH oxidized/h/100 milligram of protein.
**Influence of nigerloxin on lipid peroxides in lens**

Lipid peroxidation level, measured as TBARS in the lens of nigerloxin treated galactosemic rats is presented in Table-2. Lipid peroxides levels were significantly \((p<0.05)\) higher in the lens of galactose fed animals as compared to that of normal control animals. Nigerloxin administration at a dose of 100 mg/kg body weight, significant decreased the levels of lens TBARS compounds \((p<0.05)\) in galactose fed animals as compared to that of untreated galactose fed animals. Lipid peroxides in the lens of normal animals were not altered by nigerloxin administration.

**Influence of nigerloxin on reduced glutathione and total thiols in lens**

Effect of nigerloxin on lens GSH and total thiols are presented in Table-2. Lens GSH concentration was significantly decreased in galactose fed animals as compared to normal control animals. Nigerloxin treated animals at a dose of 100 mg/kg significantly elevated these antioxidant molecules in the lens of galactose fed animals. Total thiols concentration was also increased by nigerloxin administration. Low dose of nigerloxin did not facilitate these molecules in lens of galactose fed animals. Levels of GSH and total thiols were unaltered in normal animals administered nigerloxin as compared to normal control animals.

**Influence of nigerloxin on lens antioxidant enzymes**

Activities of antioxidant enzymes in the eye lens of galactose fed rats treated with nigerloxin is represented in Table-3. Activities of lens antioxidant enzymes – superoxide dismutase and glutathione peroxidase were decreased in galactose fed animals compared to the normal control animals. Glutathione-S-transferase activity was not altered. Administration of nigerloxin prevented the decrease in these enzyme activities in the eye lens of galactose fed animals. Enzyme activities in normal were not altered by nigerloxin treatment.
**Fig.1.A.** Influence of nigerloxin on the fluorescence of soluble protein in lens of galactose fed rats by advanced glycation end products (AGEs). Protein (1 mg/mL) in 0.05M sodium phosphate buffer pH 7.4 was excited at 370 nm and emission was monitored 400-500 nm. Data represent mean of 8 animals in each group.

**Fig.1.B.** Influence of nigerloxin on AGEs related fluorescence of soluble protein in lens of galactose fed rats. Data represent fluorescence intensity at 421 nm. All values are mean ± SEM of 8 animals in each group; a, significantly (P<0.01) different from Normal Control group; b, significantly (P<0.01) different from Galactose Control group.
Fig. 2. A. Influence of nigerloxin on the fluorescence of soluble protein in lens of galactose fed rats by tryptophan. Protein (1 mg/mL) in 0.05M sodium phosphate buffer pH 7.4 was excited at 295 nm and emission was monitored 310-400 nm. Data represent mean of 8 animals in each group.

Fig. 2. B. Influence of nigerloxin on tryptophan fluorescence of soluble protein in lens of galactose fed rats. Data represent fluorescence intensity at 339 nm. All values are mean ± SEM of 8 animals in each group; a, significantly (P<0.01) different from Normal Control group; b, significantly (P<0.01) different from Galactose Control group.
Table-2. Influence of nigerloxin on lens lipid peroxides and glutathione in galactose fed rats.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Lipid peroxides (nmol/mg protein)</th>
<th>Reduced glutathione (µg/mg protein)</th>
<th>Total thiols (mM/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose Control</td>
<td>1.58 ± 0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>22.9 ± 1.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.410 ± 0.012&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galactose + Nigerloxin (25 mg/kg)</td>
<td>1.39 ± 0.13</td>
<td>21.7 ± 1.43</td>
<td>0.399 ± 0.013</td>
</tr>
<tr>
<td>Galactose + Nigerloxin (100 mg/kg)</td>
<td>0.76 ± 0.07&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.5 ± 1.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.560 ± 0.042&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal Control</td>
<td>0.47 ± 0.04</td>
<td>41.6 ± 1.69</td>
<td>0.866 ± 0.037</td>
</tr>
<tr>
<td>Normal+ Nigerloxin (100 mg/kg)</td>
<td>0.54 ± 0.02</td>
<td>46.6 ± 3.33</td>
<td>0.849 ± 0.039</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 Galactose Control group.
Table-3. Influence of nigerloxin on lens antioxidant enzymes in galactose fed rats.

<table>
<thead>
<tr>
<th>Animal group</th>
<th>Superoxide dismutase&lt;sup&gt;1&lt;/sup&gt; (U/mg protein)</th>
<th>Glutathione-S-transferase&lt;sup&gt;2&lt;/sup&gt; (nmol/min/mg protein)</th>
<th>Glutathione peroxidase&lt;sup&gt;3&lt;/sup&gt; (nmol/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose Control</td>
<td>0.976 ± 0.051&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.8 ± 2.70</td>
<td>25.5 ± 0.67&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Galactose + Nigerloxin (25 mg/kg)</td>
<td>0.981 ± 0.019</td>
<td>26.2 ± 1.57</td>
<td>28.8 ± 1.16</td>
</tr>
<tr>
<td>Galactose + Nigerloxin (100 mg/kg)</td>
<td>1.024 ± 0.039&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.7 ± 2.35</td>
<td>30.5 ± 1.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Normal Control</td>
<td>1.158 ± 0.045</td>
<td>26.9 ± 1.99</td>
<td>32.1 ± 0.78</td>
</tr>
<tr>
<td>Normal + Nigerloxin (100 mg/kg)</td>
<td>1.251 ± 0.045</td>
<td>33.6 ± 1.21</td>
<td>31.7 ± 1.56</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 Galactose Control group.

1: One unit of activity was taken as the enzyme reaction which inhibits the rate of cytochrome C reduction by 50%.

2: Nanomole of CDNB-GSH complex formed/minute/milligram of protein.

3: Nanomole of NADPH oxidized/minute/milligram of protein.
Discussion

Numerous reports have indicated the beneficial influence of aldose reductase inhibitors (ARIs) on polyol pathway and antioxidant defense system with cataractogenesis in experimental animals as well as in clinical trials, but none have achieved clinical success for diverse reasons. Opacity of the lens is a multifactorial disorder that results in the development of cataract. Cataract is the one of the most frequent cause of visual impairment throughout the world. Intraocular elevation of polyol pathway enzyme activities and increased oxidative stress are key factors responsible in the development of hyperglycemia and galactose induced cataractogenesis. It is reported that administration of 30% galactose for a period of 23 days can effectively induce cataract in rats (Suryanarayana, et al. 2003). Galactose can readily enter the lens cell and be reduced by aldose reductase to the corresponding alcohol, galactitol via the oxidation of NADPH. Similarly, like sorbitol galactitol also accumulates inside the cell imparting osmotic stress followed by altered level of antioxidant scavengers leading to the development of cataract. In hypergalactosemic rats galactitol is not subsequently metabolized by sorbitol dehydrogenase enzyme as compared to the sorbitol. Hence accumulation of galactitol results in greater osmotic changes in the lens of galactosemic rats and induces severe degree of cataract than hyperglycemia (Kinoshita et al. 1981; Kinoshita and Nishimura, 1988; Ohta, et al. 1999; Ohta, et al. 2000). It is proposed that three major mechanisms involve in the formation of cataract − oxidative stress, polyol pathway and non-enzymatic glycation (Kinoshita, 1990). In the present study, we have focused on the alteration in polyol pathway and antioxidant defense system in the lens of galactose fed animals to assess the beneficial influence of nigerloxin.

In our previous study, we demonstrated the potential of nigerloxin to inhibit aldose reductase enzyme in experimentally induced diabetic rat lens (Suresha et al. 2012). The present investigation suggests that nigerloxin treatment significantly inhibited the elevation in aldose reductase activity in the lens of galactose fed animals. Sorbitol dehydrogenase activity was not altered by galactose feeding and nigerloxin administration.
According to oxidative stress theory free radicals play a major role in the development of cataract. Decreased antioxidant defense is well documented in diabetic cataract. Decreased activities of antioxidant enzyme (Strain, 1991) and altered level of oxidant molecule, reduced glutathione are reported in diabetes induced cataract (Obrosova and Stevens, 1999). In our previous study, we showed that administration of nigerloxin elevated activities of antioxidant enzymes – superoxide dismutase, glutathione-S-transferase and glutathione peroxidase – in the lens of diabetic animals. Reduced glutathione concentration in the lens also increased in nigerloxin administered animals thus showing its antioxidant potentials in diabetes (Suresha et al. 2012). In the present study, we report the beneficial influence of nigerloxin on the antioxidant system in the lens of galactose fed animals.

Lens superoxide anion radicals are mainly scavenged by superoxide dismutase enzyme, hydrogen peroxide and hydroperoxides toxicities are alleviated by glutathione-S-transferase enzyme and glutathione peroxidase detoxifies peroxides by using reduced glutathione. The superoxide dismutase and glutathione peroxidase activities were decreased in the lens of galactose fed animals. As a result, free radicals accumulate leading to a severe oxidative damage to the lens. Nigerloxin administration led to a restoration of these enzyme activities. Elevated activities of these enzymes may protect lens by scavenging toxic radicals.

GSH is one of most important lenticular antioxidant molecule, involved in scavenging of free radicals. GSH can act directly as an antioxidant by scavenging hydroxyl radical and singlet oxygen. It removes hydrogen peroxide and lipid peroxides by maintaining the reduced state of glutathione peroxidase sulfhydryl groups. GSH also potentiates antioxidant defense by reducing ascorbic acid to its active form (Yokoyama et al. 1993). Lens GSH concentration was decreased in galactosemic rats show an increased oxidative stress. Increased oxidative stress may utilize more amount of GSH progressively leading to its depletion in lens. Over-consumption of NADPH in polyol pathway also directly shares its depletion. The galactose induced decrease in lenticular GSH concentration is
consistent with a previous study (Lou et al. 1988). Nigerloxin administration resulted in a recovery of this molecule in lens of galactose fed animals. This influence may be due inhibition of aldose reductase, resulting in decreased usage of NADPH and increased availability of NADPH by regeneration from oxidized form.

A decrease in the antioxidant defense leads to an excess availability of superoxide anion 'O$_2^-$' and hydrogen peroxide in biological systems, resulting in increased generation of hydroxyl radicals leading to initiation and propagation of lipid peroxidation. Lens MDA levels were increased in galactose fed animals accounting for an increased generation of free radicals. Other researchers have also reported elevated lens MDA in galactose fed animals (Suryanarayana, et al. 2003). Nigerloxin reduced accumulation of these products in lens presumably by scavenging free radicals.

Reducing sugars such as glucose and galactose are capable of forming stable adducts with proteins. Protein glycation is initiated by an addition reaction between a free amino group and the carbonyl group of a sugar to form a reversible Schiff base, which undergoes rearrangement to form a Amadori products. The Amadori products can be transformed into reactive dicarbonyl products to form advanced glycation end products (AGE). Increased formations of AGEs have been implicated in the development of cataract. In this study, increased AGEs intensity reflects increased accumulation of AGEs in the lens. Administration of nigerloxin reduced the formation of AGEs. Tryptophan fluorescence was decreased in the lens of galactose fed rats that reflects protein oxidation and conformational changes. Nigerloxin administration significantly countered such modulations of protein.

In summary, a high galactose diet altered polyol pathway and antioxidant system in the lens of experimental animals. Increase in lipid peroxidation and advanced glycation end product formation in the lens of galactose fed animals were countered by nigerloxin administration. Lens aldose reductase activity was inhibited by nigerloxin. Also nigerloxin prevented deleterious influence of galactose on lens antioxidant enzymes and
GSH. These data suggest that nigerloxin has the potential of delaying or ameliorating galactose induced cataract development in rats.

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Summary

The role of osmotic and oxidative stress has been strongly implicated in the pathogenesis of cataract. Nigerloxin, a fungal metabolite, has been shown to possess aldose reductase inhibition and improved antioxidant defense system in the eye lens of diabetic rats. In the present study, the beneficial influence of nigerloxin was investigated in galactose induced cataract in experimental animals. Cataract was induced in Wistar rats by feeding 30% galactose in the diet. Groups of galactose fed rats were orally administered with nigerloxin (25 and 100 mg/kg body weight/day) for 24 days. Lens aldose reductase activity was increased significantly in galactose fed animals. Lens lipid peroxides and advanced glycation end products were also significantly increased. Antioxidant molecule – reduced glutathione, total thiols and activities of antioxidant enzymes superoxide dismutase and glutathione peroxidase were decreased in the lens of galactose fed animals. Oral administration of nigerloxin once a day for 24 days at a dose of 100 mg/kg body weight, significantly decreased lens lipid peroxides and AGEs in galactose fed rats. Lens aldose reductase activity was reduced and lens antioxidant molecules and antioxidant enzyme activities were elevated significantly by nigerloxin administration. The results suggest that alteration in polyol pathway and antioxidant defense system were countered by nigerloxin in the lens of galactose fed animals, suggesting the potential of nigerloxin in ameliorating the development of galactose induced cataract in experimental animals.

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