Soluble Lutein Protects Eye Lens for Cataract Development and Progression in Streptozotocin-Induced (STZ) Diabetic Rats

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Abstract

Background: Cataract is characterized by lens opacity induced by the cumulative or excessive exposure to light and oxygen that generate reactive oxygen species (ROS) leading to peroxidation of membrane lipids and subsequent destruction and precipitation of lens proteins. Diabetes mellitus (DM) is the primary cause of blindness. The aim of the present study was to investigate the protective effects of soluble lutein in streptozotocin-induced (STZ) diabetic rat retina for cataract development and progression.

Methods: Wistar male rats (WNIN strain, 8 weeks old) were divided into four groups [Group I, control (AIN 93, standard diet); Group II, DM (STZ); Group III: Group II + regular lutein ([Lutein and zeaxanthin, [L/Z]RL, 0.5%] and Group IV: Group II + soluble lutein [SL (Lutein and Zeaxanthin isomers [L/Zi], iLutemax 2020®, 0.5%). Animals were maintained on their respective diets for 12 weeks. DM was induced by a single intraperitoneal injection of STZ (30 mg/kg) in 0.1 M citrate buffer, pH 4.5. Cataract development and progression was evaluated by slit lamp bio-microscope examination. Lens were collected, homogenized and estimated for total and soluble protein, and protein aggregation. Malondialdehyde (MDA), protein carbonylation, and sorbitol were also determined in the lens.

Results: Results indicate that cataract score was lower in the lutein treated groups (regular or soluble form) but SL seems to be more effective than RL. SL and RL elicit antioxidant activity as evidenced by amelioration in protein carbonyls, lipid peroxidation and sorbitol levels. Fasting glucose and HbA1c levels were reduced in SL treated animals. SL prevented the aggregation of lens soluble proteins.

Conclusion: These results suggest soluble lutein was effective in reducing oxidative stress and delay cataract in hyperglycemic conditions. SL was more effective in delaying diabetic cataract compared to RL at dose of 0.5% in the diet which is reflected in molecular analysis related to cataractogenesis.

Keywords: Diabetes; Cataract; Lutein; Diabetes Mellitus

Abbreviations

SL: Soluble Lutein; RL: Regular Lutein; L/Zi: Lutein and Zeaxanthin Isomers; D: Diabetes; C: Control; TBARS: Thiobarbituric Acid; HMW: High Molecular Weight; MDA: Malondialdehyde; STZ: Streptozotocin; DM: Diabetes Mellitus; AR: Aldose Reductase; SDH: Sorbitol Dehydrogenase; LEC: Lens Epithelial Cells; TSP: Total Soluble Protein

Introduction

Diabetes mellitus (DM) is one of the most prevailing non-communicable, metabolic disorder characterized by hyperglycemia resulting from defective insulin production, resistance to insulin action or both. Prolonged exposure to chronic hyperglycemia can lead to various ailments including both vascular and nonvascular complications [1]. According to the International Diabetes Federation (IDF), more than 285 million people are already affected by DM worldwide, and this number is expected to rise to 439 million by 2030 [2,3]. Tissues such as retina, kidney, peripheral nerves and lens that are insulin independent for the uptake of glucose are affected with chronic hyperglycemia, resulting in the development of diabetic retinopathy, nephropathy, neuropathy and cataract respectively.

Cataract, defined as opacification of normally transparent eye lens that interferes with the transmission of light onto the retina and results in impairment of the vision. Cataract is the second most common complication of DM [4] and is associated with a 5-fold higher prevalence of cataracts [5]. In both developing and developed countries, diabetic cataract is considered as the major cause of blindness.
A wealth of literature reports that free-radicals are implicated in the pathogenesis of diabetic complications including cataract, it is suggested that antioxidants will offer a therapeutic approach to combat diabetic cataract. Our laboratory has been investigating the effect of dietary antioxidants on their ability to inhibit aldose reductase and/or combat oxidative stress to prevent or delay diabetic cataract. Earlier studies from our laboratory showed that lutein and curcumin delayed diabetic cataract in rats [28]. Lutein is a xanthophyll that belongs to the class of carotenoids. Though widely distributed in plant tissues, lutein is uniquely concentrated in the macula of the human eye, thus protecting the eye from the high-frequency light and reducing the risk of age-related macular degeneration and also cataract development. In another study, it was reported that rats treated with a combination of insulin and lutein showed delayed development and maturation of cataract than those treated with either lutein or insulin alone, and also could prevent the diabetes-induced depletion of glutathione [27]. Though, several dietary antioxidants are proven to be capable of quenching free-radicals their efficacy and bioavailability remain questionable. Safety assessment studies of L/Zi (Lutein and Zeaxanthin isomers) were performed and no adverse effects were reported [29]. This study is to investigate the effect of L/Zi (soluble lutein (SL), Lutemax 2020®) in comparison to regular lutein (RL) in preventing or delaying diabetic cataract in streptozotocin (STZ) induced diabetic rats.

Material and Methods

Experimental design: Two-month-old male WNIN (Wistar-NIN) rats with an average body weight of 217 ± 12g were obtained from the National Center for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad, India (NCLAS, NIN). Animals were maintained at NCLAS, NIN and kept for acclimatization in experimental room for two weeks. Diabetes was induced in overnight fasted animals by a single intraperitoneal injection of STZ (30 mg/kg) in 0.1 M citrate buffer, pH 4.5. Another set of rats, which received only vehicle, served as the control (Group I; n = 12). Fasting blood glucose levels were measured 72h after STZ injection. Animals having blood glucose levels > 150 mg/dL were considered diabetic and were divided into three groups (Group II- IV). Table 1 provides dose information for each treatment.

<table>
<thead>
<tr>
<th>Group</th>
<th>Abbreviation</th>
<th>Diet</th>
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<tr>
<td>I</td>
<td>C</td>
<td>AIN 93</td>
</tr>
<tr>
<td>II</td>
<td>D</td>
<td>AIN 93</td>
</tr>
<tr>
<td>Y</td>
<td>D+SL</td>
<td>AIN 93 with soluble lutein 0.5%</td>
</tr>
<tr>
<td>VI</td>
<td>D+RL</td>
<td>AIN 93 with regular lutein 0.5%</td>
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Table 1: Experimental groups and diets.

Investigational Product: Soluble lutein (SL), (L/Zi, Lutemax 2020®) supplied by OmniActive Health Technologies Ltd. India, Regular Lutein (RL) supplied by Kancor Ingredients Ltd., India. Marigold is a red orange crystal powder that is a characteristic odor of marigold flower. It is obtained by saponification of and thermal isomerization reaction of an extract comprising a xanthophyll extract such as marigold flower oleoresin. The isomerization reaction transforms certain of the free L to (3R, 3′S) - Z (meso-isomer), while saponification causes the release of free calcium formats in free form (lutein/zeaxanthin). Then, the mix is exposed to extraction, purification and drying under vacuum to obtain the L/Zi product. The product comprises 80% carotenoids with 67% L and 13.5% Z isomers. The isomeric distribution of zeaxanthin in the product is about 50:50 as a mixture of (3R,3′R)-β,β-carotene-3,3′-diol and (3R,3′S)-β,β-carotene-3,3′-diol, usually denoted to as zeaxanthin and meso-zeaxanthin, respectively.

Animal Care: Institutional and national (CPCSEA) guidelines for the care and use of animals were followed and all experimental procedures involving animals were approved by the IAEC (institutional animal ethical committee) of National Institute of Nutrition (Approval # P26/IAEC/NIN/2012/7/GBR/WNIN). For the entire study period, animals were housed in individual cages in a temperature (22±0C) and a humidity-controlled room with a 12-h light/dark cycle. All the animals had free access to water. Food intake daily, body weights, fasting blood glucose and plasma insulin by RIA kit (BRIT-DAE) were monitored weekly. Eyes were examined every week using a slit lamp bio-microscope and lens opacity was graded into five categories (0 - 4) as described by us earlier [28]. During the study, three animals in Group II and two animals each from Groups III and IV have died due to hyperglycemia.

Tissue collection and processing: Blood was drawn once every week from retro-orbital plexus for glucose and insulin estimation. At the end of 12 weeks, animals were sacrificed by CO2 asphyxiation and eye balls were enucleated. Lenses were dissected by posterior approach and stored at -80°C until further analysis. A 10% homogenate was prepared from 3-5 pooled lenses in 50 mM phosphate buffer, pH 7.4, and centrifuged at 15,000xg for 30 minutes at 4°C. The soluble fraction was collected and used for estimation of all the biochemical parameters mentioned in the study, except for malondialdehyde (MDA), which was determined in the total lens homogenate as described previously [28].

Biochemical estimations: Blood was collected at every two weeks till the end of experimental period. There is absolutely no difference in time intervals between the groups. The experimental period is same in all the groups of 12 - 13 weeks. The blood drawn on the day of animal sacrifice was used to analyze HbA1c and plasma lutein. Lens MDA, as thiobarbituric acid reacting substances (TBARS), protein carbonyl content, and sorbitol dehydrogenase (SDH) were determined according to the methods described previously [28]. Total, soluble and insoluble protein were assayed by Lowry method using BSA as standard.

Estimations of plasma lutein levels: Plasma lutein levels were estimated by HPLC using spherisorb C18 column (Waters, Co., 4.6 x 150 mm, 5 µm) connected to Dionex UltiMate 3000 Rapid Separation Liquid Chromatography (RSLC). The column was equilibrated with isocratic mixture of acetonitrile: dichloromethane: methanol in a ratio of 70:20:10 (v/v) at a flow rate of 0.5 ml/min at 25°C. 2 µl of plasma samples (extracted with hexane) were injected on to the column and lutein was detected at 300 - 600 nm.

Lens protein profiling by SDS-PAGE and size exclusion chromatography: Subunit profile and cross-linking of soluble proteins were analyzed on 10% SDS-polyacrylamide gels under reducing conditions. Crystallin distribution in the soluble protein fraction was performed by TSK-G4000 SW column (600 x 7.5 mm, TOSOH Co., Japan) using a HPLC system. The column was equilibrated with 0.1 M sodium phosphate buffer pH 6.7 containing 0.1M sodium chloride at a flow rate of 1 ml/min. Soluble protein samples (20 µl of 1 mg/ml solution) were loaded onto the column and protein peaks were detected at 280 nm.

Statistical analysis: Data were expressed as mean ± SEM. One-way ANOVA was used for testing statistical significance between groups of data and individual pair difference was tested using Duncan’s multiple-range test. Heterogeneity of variance was tested by the nonparametric Mann Whitney test where p < 0.05 was considered as significant.

Results
Food intake and body weights: The diabetic animals (both untreated and treated) on an average consumed excess diet than the control animals, as reported by us earlier [28]. However, the body weight of diabetic animals decreased significantly to that of the control group (Figure 1A). Dietary supplementation of regular lutein (RL) and soluble lutein (SL), to diabetic rats did not have any significant effect on body weights. The average food intake and body weights, of all the experimental groups at the end of the 12-weeks are given in figure 1A and 1B.

Figure 1: A and B - Average daily food intake and body weights of experimental rats. The data were expressed as mean ± SE. Control (Non-diabetic control); D (Diabetic control); D+RL (diabetic + regular lutein); D+SL (Diabetic + soluble lutein); ***P < 0.001

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Fasting blood glucose and glycated hemoglobin levels: The fasting blood glucose levels of untreated diabetic rats were significantly higher than that of the control rats throughout the experiment (Figure 2). Glycated haemoglobin (HbA1c) levels were significantly higher in all diabetic animals (> 6.5%) (Figure 2). Dietary supplementation of regular lutein (RL), soluble lutein (SL), to diabetic rats did not have any significant effect on fasting blood glucose and HbA1c levels.

Plasma lutein levels: Bioavailability of lutein was assessed by estimating plasma lutein content. Lutein was not detected in control and untreated diabetic animals. Lutein content in rats supplemented with SL found to be 0.07 µmoles/L of plasma, which is seven fold higher when compared with 0.01 µmoles in rats fed with RL (Figure 2).

Onset and progression of cataract: Hyperglycemia-induced cataract was noticed in experimental rats after 3 - 4 weeks following diabetes induction. Though there was no delay in the onset of cataract, there was a significant delay in the progression and maturation of cataract in animals supplemented with lutein. By the end of 10th week, animals in Group-II had stage 4 cataract, whereas in SL treated animals progression of cataract was restricted to stage 2.7. On other hand supplementation with RL also attenuated cataract progression to stage 3.1, indicating that dietary intervention with lutein delayed the progression of diabetic cataract. The data reveals that SL is effective than RL in delaying the progression of diabetic cataract. The data reveals that SL is effective than RL in delaying the progression of diabetic cataract. All the lenses in control animals appeared to be normal and free of opacities during the entire experimental period. The average cataract score of group-SL is statistically significant when compared with group-D at week -9 (p = 0.0341), week-8 (p = 0.0424), week-7 (p = 0.0461), and week-6 (p = 0.0424).

Figure 2: Effects of treatment on fasting plasma glucose, HBA1c, Lutein, delay of diabetic cataract in STZ-induced diabetic rats. The data were expressed as mean ± SE. Control (Non-diabetic control); D (Diabetic control); D+RL (diabetic + regular lutein); D+SL (Diabetic + soluble lutein) ***: p < 0.001.

Profile of lens proteins: There was a significant decrease in both total and soluble protein content in the untreated diabetic group compared with the control group (Figure 3). This could be due to a partial leakage of proteins into the aqueous humor or aggregation of proteins and in solubilization. Between SL and RL treated groups, SL significantly prevented loss of soluble protein compared to group-II (Table 2). Soluble protein fractionation on SDS-PAGE (Figure 4a) showed a high-molecular weight band of ~50 kDa in group-II, and a similar subunit pattern was observed in treatment groups. HPLC profile of soluble protein from group II demonstrated the reduced peak area in low molecular weight region and increased peak area in the high molecular weight region compared to group-I. HPLC profile revealed that dietary supplementation with SL prevented the alteration of soluble protein profile (Figure 4).

Figure 3: Biochemical Change in the Lens Associated with Soluble Lutein.

Figure 3a: Effect of different treatments on TBARs.

Figure 3b: Sorbitol levels in different treatments. Spectrofluorimetric measurement of lens sorbitol. The data were expressed as mean ± SEM. n = 6; Control (Non-diabetic control); D (Diabetic control); D+RL (diabetic + regular lutein); D+SL (diabetic + soluble lutein).

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Size exclusion chromatography on TSK-3000 HPLC column resulted in resolution of crystalline proteins of lens (Figure 4a). HPLC profile demonstrated the reduced peak area in low molecular weight region and whereas increased peak area in the high molecular weight protein region. This suggests there was a phenomenon of protein aggregation in diabetic conditions. Interventions with SL normalized the profile of TSP.

**Oxidative stress markers:** Oxidative stress in the lens was assessed by measuring protein carbonyls, TBARS and sorbitol levels. Accumulation of protein carbonyls was noticed in the untreated diabetic group compared to control group (Table 2). Dietary intervention with SL showed significant inhibition, over RL, of protein carbonyl formation compared with the untreated diabetic group. The results indicate the beneficial effect of SL over RL. The levels of TBARS in the untreated diabetic group were higher than the control group (Figure 3a). Elevated levels of sorbitol were observed in the untreated diabetic group (Figure 3b) when compared with control group. Dietary treatment with either RL or SL reduces sorbitol levels but not significantly when compared to untreated diabetic group (Figure 3b).

**Table 2:** Protein content in total and soluble fraction of lens homogenate.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total protein (mg/gm lens)</th>
<th>Soluble protein (mg/gm lens)</th>
<th>% Soluble protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>516.54 ± 9.3</td>
<td>388.77 ± 8.2</td>
<td>75.19 ± 2.2</td>
</tr>
<tr>
<td>D</td>
<td>281.34 ± 31.0***</td>
<td>128.87 ± 11.0***</td>
<td>46.87 ± 2.5***</td>
</tr>
<tr>
<td>D+RL</td>
<td>311.79 ± 7.8</td>
<td>156.55 ± 4.7</td>
<td>50.35 ± 1.9</td>
</tr>
<tr>
<td>D+SL</td>
<td>376.53 ± 20.9</td>
<td>218.78 ± 14.3 **</td>
<td>58.04 ± 1.8 *</td>
</tr>
</tbody>
</table>

The data were expressed as mean ± SEM. n=6; Control (Non-diabetic control); D (Diabetic control); D+RL (diabetic + regular lutein); D+SL (Diabetic + soluble lutein); ***: p < 0.001, **: P < 0.01 and *: P < 0.05 Vs C; ##: P < 0.01 and #: P<0.05 Vs D

SDS-PAGE protein profiling: Differences in protein distribution pattern were observed by running the lens protein samples on 12% polyacrylamide gel. 30 μg of protein was loaded per well along with molecular weight marker for SDS-PAGE (Broad range SDS-PAGE marker, BioRad). We have monitored the cross-links of lens proteins and high molecular weight (HMW) aggregates by SDS-PAGE. The SDS-electrophoretic pattern of the soluble protein fraction showed a band corresponding to aggregated proteins at ~50 kDa in group-D in relation to the group-C and with reduced band intensity in treatment groups (Figure 4).

![HPLC profile of peaks for each group.](image)

Discussion

This study is aimed to investigate the differences in soluble lutein and regular lutein in combating oxidative stress and to prevent or delay STZ-induced diabetic cataract. This study reveals that soluble lutein has more bioavailability than regular lutein. However, similar to regular lutein, soluble lutein had no hypoglycemic activity and did not elicit any effect in preventing accumulation of glycated hemoglobin. Interestingly, soluble lutein is relatively more effective in preventing TBARS accumulation and protein carbonyl formation in diabetic rat lens than regular lutein. This study revealed that soluble lutein is more efficient in delaying diabetic cataract compared to regular lutein, which partially could be mediated by anti-oxidative properties.

It was known that during cataractogenesis large amounts of insoluble protein derived from the soluble protein gets accumulated resulting in decreased total and soluble protein. However, a decrease in soluble protein was significantly prevented by addition of soluble lutein. Increased protein carbonyls and TBARS suggest an increased protein and lipid oxidation due to hyperglycemia. Oxidative stresses being an important factor in cataractogenesis, the decrease in protein carbonyls and TBARS suggests that soluble lutein exerts an antioxidant effect in addition to AR inhibitory potential. Earlier studies also reported the beneficial effects of lutein in diabetic mice [21,22]. There is an inverse relationship between macular pigment density and lens density, suggesting that the macular pigment may serve as the marker for xanthophylls in the lens [23] and macular pigment density represents an indirect measure of the amount of lutein present in the body [24-26]. Lens opacification was due to sorbitol, through the action of aldose reductase (AR) and draws water into the lens in diabetic cataract. In vitro and in vivo animal experiments strongly suggest that there is an association between increased oxidative stress and the development of cataract [30-32].

Accumulation of protein free carbonyls has been closely related to the development of both senile and diabetic cataracts [33,34]. Lutein supplementation reduced protein carbonyls and demonstrated delayed the progression of diabetic cataract. Oxidative damage to lens proteins is a major factor leading to cataract formation. Lutein is an antioxidant and thus prevents the progression of the disease and reduces TBARS. Basic and clinical research studies have shown that osmotic stress in the lens caused by sorbitol accumulation, induces apoptosis in lens epithelial cells (LEC) leading to the development of cataract [17,19]. Oxidative damage occurs indirectly as a result of polyol accumulation during diabetic cataract formation, the use of lutein may be beneficial to protect lens from free radical damage. Based on these results, it appears that soluble lutein was effective against osmotic stress caused by hyperglycemia.

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The presence of lutein in human tissues is of entirely dietary origin. Since soluble lutein is effective in managing oxidative stress and delay cataract under hyperglycemic conditions, lutein-rich food items such as green leafy vegetables and fruits such as kale, spinach, collards, broccoli, yellow carrots, corn, green peas and oranges could be an effective diet-based approach to supplement lutein with an increased bioavailability. Dietary antioxidants could be an effective and inexpensive approach to delay or prevent diabetic cataract.

Acknowledgments

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Conflict of Interest

The authors PYR, PSC and GBRP have no conflict of interest. JS, JD and VJ are employees of OmniActive Health Technologies.

Bibliography


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