PROTECTIVE ACTION OF CURCUMIN AND VITAMIN E AGAINST THE IMBALANCE OF ELECTROLYTES CALCIUM, SODIUM AND POTASSIUM IN GALACTOSE-INDUCED CATARACT

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ABSTRACT: Cataract is a clouding that develops in the crystalline lens of the eye leading to opacity. In this study we investigated the efficiency of curcumin and Vitamin E to regulate the altered levels of Ca²⁺, Na⁺ and K⁺ ions in cataract induced rat lens. Also specific activity of aldose reductase and the total and soluble lenticular protein levels in the lens were compared in treated as well as control groups of rats. The data was analyzed using one-way ANOVA, followed by post hoc test multiple comparisons and Karl Pearson’s correlation. Feeding of rats with Curcumin and Vitamin E had resulted in a significant decrease (p<0.05) in Na⁺ and Ca²⁺ concentrations and an increase (p<0.05) in K⁺ concentration which is otherwise found to be raised in cataractous condition. Also, a decrease in the specific activity of Aldose Reductase was found along with the conservation of total and soluble lenticular protein levels in lens treated with Curcumin and Vitamin E compared to cataract induced lens.

Key words: CURCUMIN, Vitamin E, Electrolytes, Galactose
INTRODUCTION

Cataract, loss of lens transparency, results in partial or total blindness of the eye. Galactose, a cataractogenic substance found in dairy products, has been used to induce cataract in our present study. Osmotic swelling with lens fibre membrane damage and increased uptake of sodium and chloride ions is known to occur in galactose-induced cataract (Kinoshita, 1964 et al). Understanding the role of various inorganic ions in cataract formation constitutes an important area of thrust in the field of eye research (Jedzimak, J.A et al 1976, Stanojevic, P.A et al 1987, Ringvold, A et al 1998).

Recent reports indicate that excessive Ca\(^{2+}\) can induce the formation of protein aggregates in lens homogenates and that high molecular weight aggregates may lead to lens opacification (Giblin, F.J. et al 1984). A prolonged increase of the Ca\(^{2+}\) concentration would be expected to activate proteases, such as calpain, and could induce unexpected and irreversible breakdown of important structural proteins (Duncan, G et al, 1999; Marcantonio, J.M et al, 1986; Sanderson, J et al, 1996; Pou, H. et al, 1974).

In lenses, when K\(^{+}\) is pumped into the lens, Na\(^{+}\) is pumped out generating a chemical gradient and this mechanism while regulating water content it allows the lens to act as a “perfect osmometer” and contributing to the transparency of the lens (Xiaolin, H et al 1997). In cataractous condition, influx of Na\(^{+}\) attracts water and chlorine ions paving way for disturbance of the osmotic balance in the lens environment (Stanojevic, P.A et al 1987). In addition, previous reports indicate that accumulation of Ca\(^{2+}\) and Na\(^{+}\) and loss of potassium lead to the impaired permeability of eye lens membrane resulting in the formation of cataract (Xiaolin, H et al 1997; Shukla, N et al 1996).

The present investigation aims to evaluate the alterations in the levels of Ca\(^{2+}\), Na\(^{+}\) and K\(^{+}\) in the galactose induced cataractous lenses with an emphasis on the defensive role of curcumin and vitamin E combination on these important ionic variations.

Materials and Methods

Materials

Galactose, curcumin, and bovine serum albumin (BSA) were obtained from Sigma Chemical (St. Louis, MO, USA). All chemicals and solutions are of analytical grade unless otherwise specified.
Experimental Design
Male Wistar-NIN rats (21-24 days) having an average bodyweight of 35-40g, were obtained from National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad and randomly assigned into 4 groups of 8-10 animals in each group. Each group was fed on a different diet as follows: group A: normal stock diet AIN-93 (n=8); group B: diet AIN-93 with 30% galactose (n=8); group C: group-II+ 0.01% curcumin + 15mg Vit-E/100gm diet (n=8); group D: group-II+ 0.005% curcumin + 15mg Vit-E/100gm diet (n=8)
Parallel controls were maintained for curcumin (both levels) and vitamin-E with normal stock AIN-93 diet.

Animal Care
The Departmental Animal Ethics Committee approved the safety of the animals and protocols accordingly. Rats were caged individually in a temperature- and humidity-controlled room with a 12-h light/dark cycle. The experiment was carried out for 26 days. All the rats had free access to water. Food intake (daily) and body weight (weekly) were monitored.

Tissue Collection
At the end of the experiment, rats were sacrificed by CO2 asphyxiation and lenses were extracted and stored at −80°C till the analysis was carried out.

Atomic Absorption Spectrophotometric Studies
Pooled lens (of each group) were individually placed in 1 ml of concentric nitric acid (70% v/v), allowed to dissolve overnight and subsequently rota mixed. A volume of 0.2 ml of the dissolved tissue was placed in a test tube containing 9.8 ml of deionised water and rota mixed. This solution was subsequently used to measure the levels of Ca²⁺ Na⁺ and K⁺ using GBC Atomic Absorption spectroscopy (GBC Scientific Equipment Pvt. Limited, Australia 3175) according to established methods (Mata, A.D, 2004; Bracken, N.Ket al ,2002) against appropriate standards (Table I).

<table>
<thead>
<tr>
<th>Element</th>
<th>Wavelengths (nm)</th>
<th>Fuel</th>
<th>Slit (nm)</th>
<th>Sensitivity (µg/ml)</th>
<th>Optimum working range (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca²⁺</td>
<td>422.7</td>
<td>AAc</td>
<td>0.5</td>
<td>0.02</td>
<td>5-20</td>
</tr>
<tr>
<td>Na⁺</td>
<td>586.6</td>
<td>AAc</td>
<td>0.5</td>
<td>0.008</td>
<td>0.4-1.5</td>
</tr>
<tr>
<td>K⁺</td>
<td>769.9</td>
<td>AAc</td>
<td>0.5</td>
<td>0.024</td>
<td>1.1-4.4</td>
</tr>
</tbody>
</table>
Aldose Reductase and Protein Content

Aldose reductase activity was estimated according to Hyman et al (Hayman S & Kinoshita J H 1965). The protein content of lens (total and soluble) was estimated as per Lowry et al. (Lowry, O. H; 1951) using BSA as the standard.

Statistical Analysis

The statistical analysis involved several tests, such as differences between the control and treated groups were analyzed using one-way ANOVA, followed by post hoc test multiple comparisons and Karl Pearson’s correlation.

RESULTS

Food Intake and Body Weight
During the entire course of the study, there was no significant variation in food intake. A significant decrease (p<0.05) in body weight in group II was observed in comparison with group I (data not shown).

Protein Content
There was a significant decrease in both total and soluble lenticular protein in group II rats compared to group I. Feeding of curcumin and vitamin E improved both the total and soluble protein levels in relation to group II. The ability of curcumin and Vitamin E to prevent the loss of soluble protein of lens tissue in groups III to VII rats was significant (Table II)

Table II: Effect of Curcumin and Vitamin-E on lenticular protein content and aldose reductase activity in galactose-induced rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein (mg/lens)</th>
<th>Soluble protein (mg/lens)</th>
<th>Aldose Reductase (Units/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Cont.)</td>
<td>15.46±0.94</td>
<td>11.30±1.74</td>
<td>22.49±0.526</td>
</tr>
<tr>
<td>II (30% Gal.)</td>
<td>8.65±1.32</td>
<td>4.96±0.99</td>
<td>37.54±0.443</td>
</tr>
<tr>
<td>III (30% gal. + 0.01% cur.+ 15 mg VE/100gm diet)</td>
<td>14.41±0.84</td>
<td>10.55±0.59</td>
<td>26.31±0.782</td>
</tr>
<tr>
<td>IV (30% gal. + 0.005% cur. + 15mg VE/100gm diet)</td>
<td>13.49±1.81</td>
<td>9.47±0.51</td>
<td>28.51±0.585</td>
</tr>
</tbody>
</table>
Aldose Reductase
The specific activity of AR, a key enzyme of the polyol pathway, was significantly higher in lenticular tissue of group II animals than group I, and feeding of curcumin and vitamin E resulted in a significant decrease in AR activity in relation to galactose-fed rats (group II) (Table I)

Element Analysis
Ca\(^{2+}\) and Na\(^{+}\) concentrations were significantly higher (p<0.05), whereas K\(^{+}\) was lower in group II lenses in relation to controls. Curcumin and Vitamin E treatment has resulted in a significant decrease (p<0.05) in Na\(^{+}\) and Ca\(^{2+}\) concentrations and an increase (p<0.05) in K\(^{+}\) concentration in comparison to the galactose-fed group II. A significant correlation between Ca\(^{2+}\) and Na\(^{+}\) was noticed in controls, in group II, and in groups III to VII. However, the concentration of K\(^{+}\) showed a decrease, and an inverse correlation was observed between Ca\(^{2+}\) and K\(^{+}\) in galactose-fed group II rats (r=−0.962; p<0.05), suggesting an increased influx of Ca\(^{2+}\) into the lens with underlying implications on opacification.

Discussion
In the present study, aldose reductase activity had significantly increased in Group II compared to Group I. Aldose reductase catalyses the reaction of galactose with NADPH leading to the formation of galactitol, a sugar alcohol, accumulation of which is the initiating factor in galactose induced cataract. As galactitol is not further metabolized nor does it readily diffuse through lens membrane, it accumulates water in the lens leading to osmotic swelling with lens fibre membrane damage (Kinoshita, J.H. et al ,1974; Kinoshita, J.H. 1965) which in turn allows Na\(^{+}\) to enter the cell and causes further water absorption resulting in intracellular content leak out, releasing K\(^{+}\) and other proteins of the lens (Gabbay, K.H. (1973) . In accordance with the above studies, an elevated Na\(^{+}\) and reduced K\(^{+}\) concentrations have been observed suggesting the aldose reductase mediated osmotic disequilibrium in galactose fed Group II.
Ca\(^{2+}\) is an important ion particularly with reference to its involvement in the mechanism of cataractogenesis. In lenses, the concentration of Ca\(^{2+}\) also represents an important factor for the permeability of the membrane to Na\(^{+}\) ions (Hightower, K.R ,1982). In the present study, Ca\(^{2+}\) has markedly increased along with an imbalance in Na\(^{+}\) and K\(^{+}\) concentrations in the cataractous lenses of Group II. This observation is in compliance with the earlier studies, which have indicated that an increase of Na\(^{+}\) and a loss of K\(^{+}\) do not produce lens opacity if the Ca\(^{2+}\) concentration remains within normal values. On the other hand, an increase in Ca\(^{2+}\) concentration, coupled with an imbalance of Na\(^{+}\) and K\(^{+}\), may prove detrimental (Rac, Z.P, 1977; Cekic, O. (1998,; Hightower, K.Ret al ,1982).
Curcumin and vitamin E inclusion in Group III-VII resulted in a significant decrease in Ca\(^{2+}\) and Na\(^{+}\) and increase in K\(^{+}\). This suggests that curcumin and vitamin E by mechanism(s) not fully understood at this present time, prevents polyol accumulation mediated through the inhibition aldose reductase and there by restoring the osmotic equilibrium by the normalization of Na\(^{+}\) and K\(^{+}\) levels to a great extent and hindering the influx of Ca\(^{2+}\) and thus preventing the lens from becoming opaque as evidenced by the curcumin and vitamin E combination treated Group III-VII rats vis-à-vis the galactose treated group II. The synthesis of crystalline or soluble proteins by the lens fiber cells is also markedly reduced in the osmotic cataracts associated with ionic imbalance (Dische, Z et al 19 56; Shinohara, T.et al, 1982; Kador, P.F et al ,1979). Previous studies on diabetic rats have showed that increased levels of Ca\(^{2+}\) in lens could lead to over-activation of tissue specific proteases, resulting in the degradation of lens proteins (Azuma, M. et al ,2004). In agreement with these studies, total and soluble proteins of the lenses of Group II animals were greatly reduced along with an increase in Ca\(^{2+}\) levels. This could be due to increased protein degradation mediated by increased protease activity. Normalization of the protein content with the treatment of curcumin and vitamin E combination was observed in Group III-VII rats and this could perhaps be attributed to an inhibition of Ca\(^{2+}\) induced protease activity of the lens.

**Conclusion:**

Our study suggests that curcumin and vitamin E combination not only prevents a gamut of alterations imposed by galactose stress, but also was found to normalize various important cations that are implicated in changing the lens protein profile and thus contributing to the transparency of the lens.

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REFERENCES


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