Original Article

Selenite Cataract and its Attenuation by Vitamin E in Wistar Rats

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Purpose: To study the role of vitamin E in preventing cataract formation in experimental animals.
Methods: An experimental model (selenite cataract) was selected for this study. Selenite cataract was produced in rats by subcutaneous administration of sodium selenite. Biochemical and histological changes following induction of selenite cataract in weanling wistar rats were studied vis-à-vis the role of vitamin E in attenuating or preventing cataractogenesis.
Results: Vitamin E was capable of preventing selenite cataractogenesis. Selenite cataract did not develop in 91.6% (11 of 12) and 76.7% (8 of 12) vitamin E treated rats, when administered on the 12th and 10th post partum day respectively.
Conclusion: The study confirmed that selenite induced cataract in wistar rats is attenuated by vitamin E.

Keywords : Selenite cataract, vitamin E, Wistar rats

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The cataractogenic potency of selenite was first reported in the early 50s by Algana and D' Aquino.[1] The selenite cataract model is an appropriate specimen for the study of cataractogenesis, since it exhibits many of the morphological changes observed in senile cataract. Rats of different species and from different origins, display varied susceptibility to formation of selenite cataract. Devamanoharan et al[2] reported 15% cataract on the 15th day of experiment after 0.3 mmol ascorbate and selenite treatment in Sprague-Dawley rats, while Orhan et al[3] observed 41.6% cataract on the same day of the experiment in Wistar albino rats.

Administration of selenite increases peroxidation of lens lipids as well as formation of H_2O_2 in the aqueous humour. This disturbance of oxidative stress is corrected by various antioxidants. These include sodium ascorbate (Vitamin C[2]); Pantethine, Glutathione isopropyl ester, Glutathione, L-ascorbic acid phosphate, Cysteamine S-Phosphate4; WR _ 77913; an amino phosphorothioate[5], Deferoxamine[6]; Propolis, Diclofenac, Vitamin C, Quercetin[3]; Naproxen[7]; and S-diethylsuccinyl glutathione isopropyl ester.[8] Vitamin-E has been found to inhibit lipid peroxidation and cataract formation in organ culture[9] in experimental animal models *in vivo*[10] and in corticosteroid-induced cataract.[11,12] But, the antioxidant property of vitamin E has not been examined in selenite cataract. The present study was designed to test the anti-cataractogenic property of Vitamin E in selenite cataract.[13-15]

The study had three objectives: 1) to observe the histological changes in the lens after administration of selenite in wistar albino rats; 2) to study the anti-cataractogenic potency of vitamin E; and 3) to analyse the biochemical status of the lens after combined selenite and vitamin E treatment.

Materials and Methods

The anticataractogenic effect of vitamin E was studied on a single dosage of sodium selenite (32µg/~20g rat pups) subcutaneously with a 2.50mg vitamin E (α-tocopherol acetate)/rat pups subcutaneously. Eighteen rat pups were used in the study. They were 10 or 12 days old, weighing approximately 20 gm. Twelve rat pups were used for this study and 12 rat pups served as controls.

The protocol was as follows. Selenite was administered on the 10th or 12th day post partum and ten minutes later, vitamin E was administered at another site. Five doses of vitamin E (1 to 5 days) were given on consecutive days (Table 1). The frequency of cataractogenesis was tabulated. In all cases, non-vitamin E treated selenite injected rat pups of the same age and size served as controls. For selenite cataractous cases, vehicle-treated rat pups of the same age served as controls. Biochemical, histomorphological and electrophoretic analysis

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were done to understand the structural and biochemical status of the lens after selenite and vitamin E administration.

The experimental animals and their controls were anaesthetised, and the complete eye lenses were dissected out by the posterior approach at the required time (Table 1). The enucleated eye lenses were immediately blot dried on a blotting paper and weighed for biochemical studies using standard protocols. These include estimation of protein Glutathione, Ascorbic acid, Sulfhydryl groups, Malondialdehyde, Adenosine triphosphatase, Acid phosphatase, g-Glutamyl Cysteinyl Synthetase, Glutathione reductase, and Ion analysis.[16-25]

The dissected lens samples were used to prepare homogenate to test the biochemical parameters. In brief, for total protein estimation the lens homogenate was prepared in 5% Trichloroacetic acid. The precipitated protein was dissolved in Sodium hydroxide and used as aliquots for the estimation of total proteins. Soluble and insoluble fractions of the protein were estimated by preparing homogenate in double distilled water. The water soluble supernatant was used for estimation of soluble protein and the residue was dissolved in sodium hydroxide and used for the estimation of insoluble protein. Glutathione estimation was carried by preparing lens homogenate in 3% Metaphosphoric acid. For the estimation of Sulfhydryl groups the lens homogenate was prepared in 0.02 M EDTA at 4°C. Ascorbic acid estimation was carried out by preparing lens homogenate in Norit reagent. For the estimation of MDA lens homogenate was prepared in ice cold 0.025 M Tris-Hcl. Sodium, Potassium, Calcium and Magnesium were analysed by digesting the lens samples to dryness in Nitric acid and Hydrogen peroxide. The resulting residual salt (ash) was dissolved in 1% Hydrochloric acid containing 0.2% Lanthanum chloride and used for the elemental analysis by atomic absorption spectroscopy. Analysis of ATPase and Acid phosphatase activity was done after preparing lens homogenate in double distilled water. For g-Glutamyl cysteinyl synthetase assay, the lens homogenate was prepared in ice cold 0.02M Tris buffer at 4°C and the supernatant used for the assay. Glutathione reductase was assayed after preparing the homogenate in chilled Phosphate buffer, and the supernatant used for the assay. Each estimations were done 12 times. The results were expressed as mean ± S.D. The results obtained were

Table I. Experimental design

Group	No of animals	Na_2SeO_3 dosage in (mcg)	No. of doses of Na_2SeO_3	Vit - E dosage in mg	No. of doses of Vit-E	Administration started on post partum days	Cataract incidence% on both eyes	Stage of observation after Administration
I A Selenite alone- (Test)	12	32	1	0	0	10 or 12	100	1,2,3,4 and 5th day Nuclear, Mature amd Hypermature
I B Selenite alone (Control)	12	0	0	0	0	10 or 12	0	1,2,3,4 and 5th day and subsequent days up to 30th day
II A Selenite + Vit. E (Test)	12	32	1	2.50	1	10	100	1,2,3,4 and 5th day and subsequent days up to 30th day
	12	32	1	2.50	2	10	100	
	12	32	1	2.50	3	10	100	
	12	32	1	2.50	4	10	83.33 ± 2.00	
	12	32	1	2.50	5	10	33.32 ± 4.00	
II B Selenite + Vit. E (Control)	12	32	1	0	0	10	100	1,2,3,4 and 5th day and subsequent days up to 30th day
III A Selenite + Vit. E (Test)	12	32	1	2.50	1	12	100	1,2,3,4 and 5th day and subsequent days up to 30th day
	12	32	1	2.50	2	12	83.33 ± 2.00	
	12	32	1	2.50	3	12	75.00 ± 4.00	
	12	32	1	2.50	4	12	50.00 ± 2.00	
	12	32	1	2.50	5	12	8.33 ± 1.00	
III B Selenite + Vit. E (Control)	12	32	1	0	0	12	100	1,2,3,4 and 5th day and subsequent days up to 30th day

statistically analysed using one way analysis of variation (ANOVA) and t test (Snedcor and Cohran[26]). P-values of <0.01 and <0.05 were considered statistically significant. These values were considered highly significant based on the increase in the F values of each parameter. The statistical analysis was done by comparing selenite alone treated cataractous case with normal lens (control) and Selenite + vitamin E treated non-cataractous case with selenite treated cataractous case (control).

Qualitative analysis of the protein was done using SDS-PAGE as described by Laemmli.[27] The proteins were separated on a mini vertical slab gel (Broviga) electrophoretic apparatus. Four weighed lens samples from different stages after selenite administration (i.e., 3rd day, 5th day, nuclear, mature, stages and their controls) were homogenized in 0.5ml of Phosphate buffered saline (PBS) solution (pH 7.4), centrifuged at 12,000g for 10 minutes. The supernatant was designated as the water-soluble protein fraction. The residue was washed in PBS solution (pH 7.4) three times and centrifuged at 12,000g for ten minutes. The residue was dissolved in 20ml of 10% SDS, designated as water insoluble protein fraction. The proteins were dissolved in sample buffer in the ratio 1:1 and incubated in a boiling water bath for two to three minutes and chilled for one minute, then brought to room temperature. The sample was used immediately. The electrophoresis was done at a constant voltage of 50V for 20 minutes followed by 100V for 5 to 6 hours on a 12% SDS PAGE system. Standard SDS molecular weight markers were used. After electrophoresis the gel was developed, using silver staining protocols.

Table 2. Biochemical values of studied parameters

No.	Parameters	Normal lens	Selenite-treated cataractous lens (control)	Selenite + Vit.E treated non-cataractous lens
1	Total Protein (mg/mg wt.)	0.361 ± 0.003	0.336 ± 0.012	0.356 ± 0.014
2	Soluble Protein (mg/mg wt.)	0.282 ± 0.005	0.180 ± 0.001**	0.248 ± 0.004**
3	Insoluble Protein (mg/mg wt.)	0.070 ± 0.002	0.136 ± 0.001**	0.102 ± 0.002**
4	Glutathione (µg/mg wt.)	2.40 ± 0.05	1.39 ± 0.01**	2.19 ± 0.01**
5	Total Sulfhydryl Groups (µM/g wt.)	59.69 ± 0.40	39.17 ± 0.15**	51.34 ± 0.60**
6	Non Protein Sulfhydryl Groups (µM/g wt.)	7.71 ± 0.20	4.57 ± 0.02**	6.62 ± 0.08**
7	Protein bound Sulfhydryl Groups (µM/g wt.)	51.98 ± 0.40	34.63 ± 0.25**	45.42 ± 0.57**
8	Ascorbic Acid (µg/100mg)	2.32 ± 0.04	1.72 ± 0.02**	2.01 ± 0.01**
9	Malondialdehyde (µM/100g)	0.1334 ± 0.0037	0.4568 ± 0.0003**	0.1107 ± 0.0003**
10	Adenosine triphosphatases (µM ip/100mg/hr)	4.24 ± 0.04	3.75 ± 0.05**	4.07 ± 0.04**
11	Acid Phosphatases (µM p-nitro phenyl released/100mg wt.)	0.93 ± 0.03	1.45 ± 0.04**	0.93 ± 0.03**
12	g Glutamyl cysteniyl synthetase (µM ip/gm/hr)	1.69 ± 0.08	1.25 ± 0.01**	1.50 ± 0.04 **
13	Glutathione reductase (U/mg)	0.29 ± 0.04	0.14 ± 0.01**	0.23 ± 0.02**
14	Sodium (% wt.)	0.196 ± 0.001	0.315 ± 0.002**	0.263 ± 0.004**
15	Potassium (% wt.)	0.565 ± 0.002	0.954 ± 0.002**	0.896 ± 0.002**
16	Magnesium (% wt.)	0.098 ± 0.005	0.043 ± 0.001**	0.046 ± 0.004**
17	Calcium (% wt.)	0.014 ± 0.004	0.025 ± 0.002**	0.016 ± 0.001**

*n = 12; **P < 0.001; *P < 0.05; All values are mean ± S.D.*

For histological studies using light microscopy (LM) the enucleated lens were immediately fixed in Carnoy_s fixative at room temperature. For scanning electron microscopic (SEM) studies the dissected lens were immediately fixed in three percent glutaraldehyde in 0.1 M phosphate buffer at 4°C. One hour after fixation, the lenses were cut into equal halves, anterio-posteriorly or meridonally and kept in the fixative overnight. Fixed lenses were transferred to graded ethyl alcohol for LM, cleared in xylene and embedded in paraffin wax at 58°C. Sections were cut no thicker and stained with haematoxylin-eosin. For SEM Studies, after fixation the lenses were dehydrated in graded acetone series and critical point dried. The lenses were then coated with gold and used for the SEM study under a Philips scanning electron microscope 501(B) at 50 kV, and suitable photographs were taken.

Microphotographs were taken using a Pentax camera attached to the (Olympus) micrsoscope. External photographs were taken with the aid of macro lenses (52 mm x 2) attached to a Canon EOS 650 (50 mm., 1:1.8) camera.

Results

Anticataractogenic and Biochemical studies

The rat pups injected with selenite alone produced nuclear cataract on the 16th day post partum. The selenite + vitamin E injected group showed 8.33 % (1 of 12) incidence of cataract after the final dose of vitamin E, when the experiment started on 12th day post partum and the incidence of cataract was 33.3% (4 of 12) when given on 10th day post partum (Table 1). The biochemical parameters of Selenite+Vitamin E treated non cataractous lenses showed an almost normal value when compared to normal lens (Table 2). These include the different forms of Protein, Sulfhydryl groups, enzymes like ATPases, acid phosphatases, g - glutamyl cysteniyl synthetase, antioxidants of the lens like glutathione, ascorbic acid and glutathione reductase and ions like sodium, potassium, calcium and magnesium. Malondialdehyde (MDA) was measured as an indicator to lipid peroxidation, which was significantly lower in the vitamin E treated group. Since selenite cataract begins with the oxidation of membrane sulfhydryl groups, which leads to alteration of membrane permeability and protein insolubilisation; the biochemical studies revealed that vitamin-E treated lens is protected from such alterations.

Histomorphological studies

To analyse the pre-cataractous changes during selenite cataractogenesis. Light microscopic studies were done each day after selenite injection up to the fifth day. Notable morphological change under light microscope was observed 3 days after selenite injection. On the third day a mild cloudy nuclear region was observed with the whole lens under light microscope. At this stage an anterio-posterior section revealed a differentiated circular adult nuclear area (Figure 1). At five days, after injection, the cloudy nuclear area showed pinhead opacity, which could be visible to the naked eye.

A double-layered epithelial layer was observed at the nuclear stage, which developed into a hyperplasia, by epithelial proliferation (Figures 2 and 3). At this stage the nuclear area showed completely damaged fibre cells, with numerous vacuoles, representing a honeycomb network (Figure 4). Arrows in Figure 4 shows the numerous minute vacuoles formed during nuclear cataract. In the cortical regions close to the nuclear area, the fibres showed areas of damage with derangement of the fibres from their normal configuration (Figure 5).

Vitamin-E+selenite-treated lenses showed normal lens morphology with typical lens nuclear and cortical fibre characteristics (Figure 6-9). An anterio-posterior section of a normal eye of vitamin E + selenite treated lens shows that the epithelium is undisturbed and observed as an intact layer (Figure 6). The nuclear fibres are densely packed in the innermost nuclear area (Figures 7 and 8). The cortical fibres are cylindrical in shape with the typical ball and socket surface structures (Figure 9).

Electrophoretic studies

Figure 10, represents the protein profile of the normal (control) [lanes 2 and 3], nuclear [lanes 4 and 5] and mature stages [lanes 6 and 7] of the selenite cataract. An increase in protein bands among SDS soluble (insoluble) fraction was observed at 45 kDa (lane 4, arrow) in the nuclear stage, when compared to the control. In the mature stage, an increase in protein bands was observed between 14 and 18 kDa (lane 6, arrows) when compared to the control. When compared to the control, the mature and nuclear stage showed a loss of protein bands among the s1oluble fraction, between 14 and 18 kDa (lanes 5 and 7, arrows).

Figure 11 represents lens protein three days after the selenite treatment [lanes 2 and 3], five days after selenite treatment [lanes 4 and 5] and vitamin-E treated non-cataractous lens [lanes 6 and 7] protein profiles. When compared to vitamin-E+selenite treated non-cataractous lens SDS soluble (insoluble) proteins, the three days and the five days selenite-treated lenses showed additional protein bands between 14 and 18 kDa, and between 18 and 24 kDa (lanes 2 and 4, arrows). The soluble protein profile showed loss of a protein band between 24 and 34 kDa (lanes 3 and 5, arrows), in lenses three days and five days after the selenite treatment, when compared to vitamin-E + selenite-treated non-cataractous lenses.

Discussion

Lipid peroxides are capable of altering membarane permeability and cell function by introducing hydrophilic moieties into the membrane hydrophobic phase.[28] The phospholipid ratio is one of many factors which govern membrane fluidity.The fluidity of the membrane is

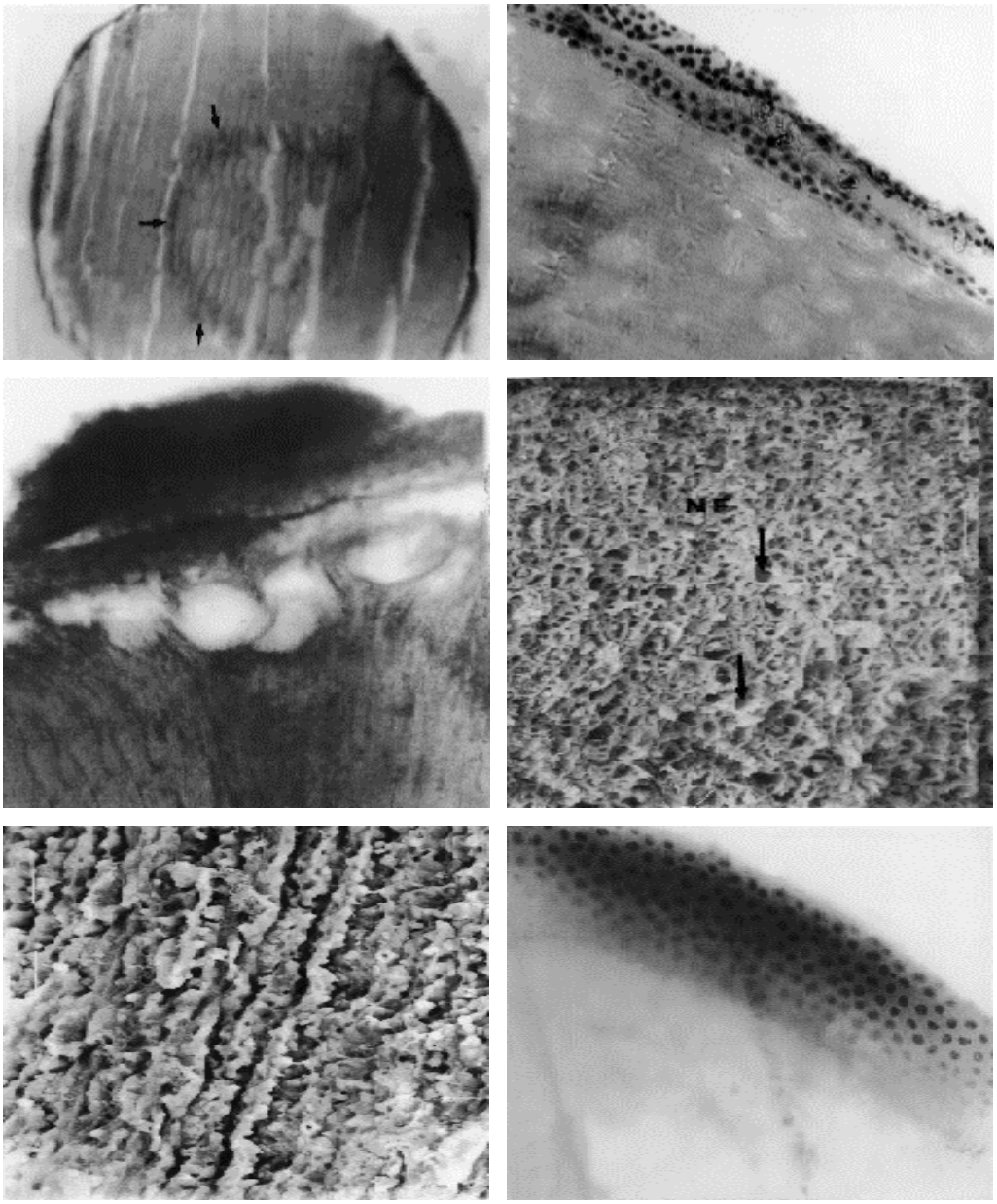

Figure 1. Third day pre-cataractous changes;anterior-posterior section showing differentiated circular area in the nuclear region (Arrows). The fibres in the centre are seen swollen (x 50)**; Figure 2.** An abnormal double layered epithelial layer observed during nuclear cataract (x 150)**; Figure 3.** Nuclear cataract;anterior-posterior section showing the highly proliferated epithelium (hyperplasia) with vacuoles formed at the anterior region of the cortical layer (x 150)**; Figure 4.** SEM photographs of nuclear cataract showing completely damaged nuclear fiber cells with numerous minute vacuoles, representing a honey comb like network (x 875) (Arrows shows the numerous minute vacuoles formed during nuclear cataract.); **Figure 5.** SEM photographs of nuclear cataract cortical fibres showing the distorted fibers with altered and damaged ball and socket surface structures (x 875)**; Figure 6.** Vitamin E and selenite treated lens; anterior - posterior section showing intact epithelium (x 150).

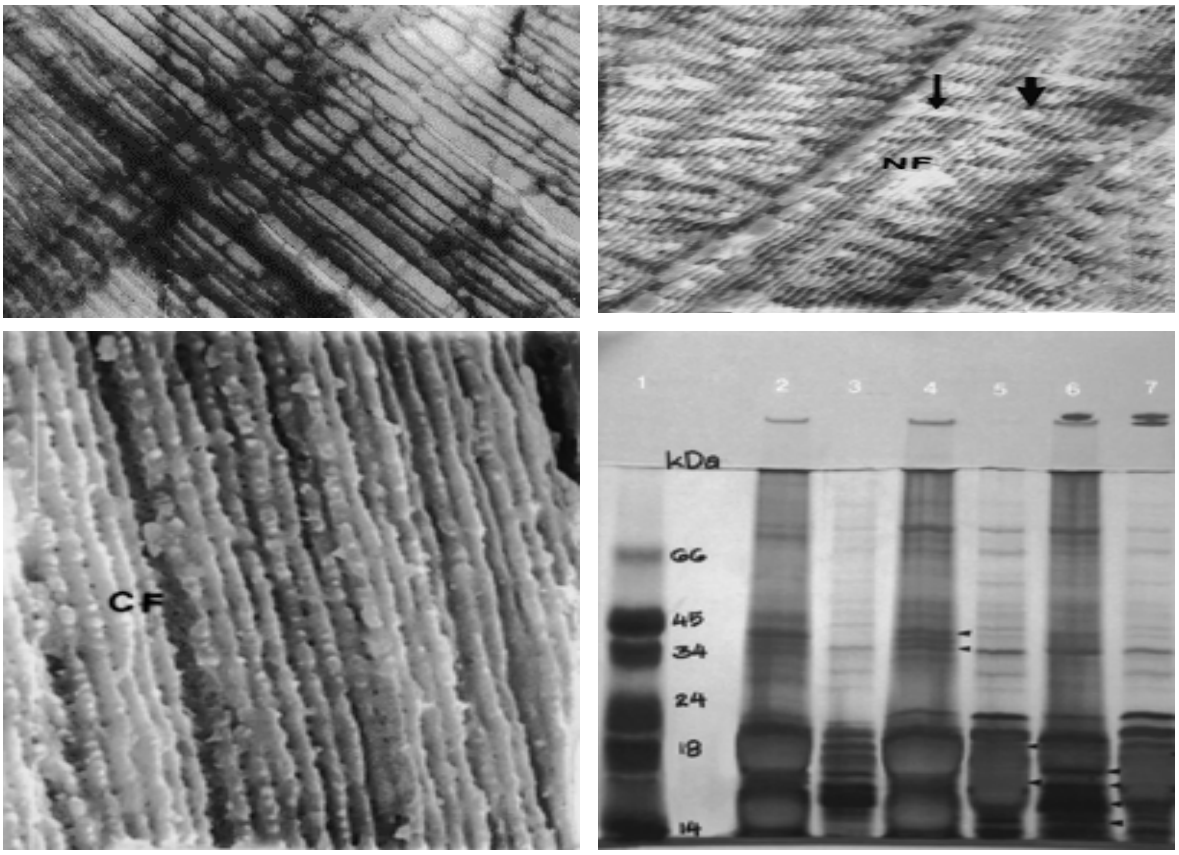

Figure 7. Vitamin E + selenite treated lens showing the normal architecture of the nuclear fibres (x 150); **Figure 8.** SEM photographs of nuclear region of Vitamin E + Selenite treated lens (x 875); **Figure 9**. SEM photographs of cortical region of Vitamin E + Selenite treated lens (x 875); **Figure 10**. SDS-PAGE showing protein profiles of normal (control) [lanes 2 and 3], nuclear [lanes 4 and 5] and mature cataract [lanes 6 and 7] stages of selenite cataract (Lanes 2,4 and 6 represent SDS soluble protein fractions range 14-66 kDa. [Arrowheads in lanes 4 and 6 shows additional insoluble protein bands observed at nuclear and mature stages; Lanes 3, 5 and 7 represent water soluable protein fractions). Lane 1 represents standard protein markers (Sigma) in the of selenite cataract at 45 kDa and in between 14 and 18 kDa mol.wt. Similarly arrowheads in lanes 5 and 7 shows the region of soluble protein loss observed at nuclear and mature cataract stages of selenite cataract in between 14 and 18 kDa mol.wt)].

responsible for the functioning of the membrane bound enzymes and the various transport process.[28] Because of oxidative insult membrane leakage occurs, leading to the degradation and insolubilization of proteins and weakening of the antioxidant system in senile cataract. That the biochemical parameters, lens histology and SDS PAGE protein did not change in the non cataractous lenses of selenite+vitamin E treated groups suggest that vitamin E is capable of preventing cataractogenic changes in selenite-administered Wistar rats.

Selenite cataract is initiated by the oxidation of the lens membrane sulfhydryl (SH) groups by the action of selenite. This mainly inactivates the Ca-ATPases and alters the ion homeostasis. The influx of calcium activates protease, Calpain II, which partially degrades lens-specific proteins a and b-crystallins. These partially degraded crystallins expose the hydrophobic regions and the thiol groups, which are then oxidised by selenite[29] or by ROS and lipid peroxides generated in the lens, to form insoluble proteins and

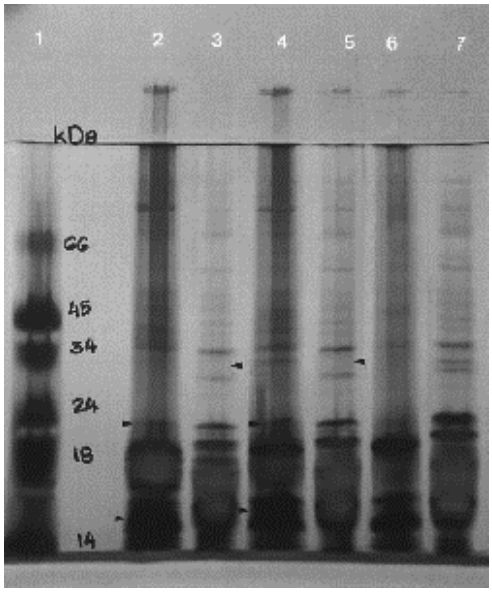

Figure 11. SDS-PAGE showing of 3days [lanes 2 and 3], 5 days after selenite treatment [lanes 4 and 5] and Vitamin E + Selenite treated non-cataractous lens protein [lanes 6 and 7] profiles (lanes 2,4 and 6 represent SDS soluble protein fractions; lanes 3,5 and 7 represent water soluable protein fractions). Lane 1 represents standard protein markers (Sigma) in the range 14-66 kDa. (Arrowheads in lanes 2 and 4 shows additional insoluble protein bands observed at 3 and 5 days after selenite administration between 14 and 18kDa and in between 18 and 24 kDa mol.wt. Similarly arrowheads in lanes 3 and 5 shows the region of soluble protein loss observed at 3 and 5 days after selenite administration in between 24 and 34 kDa mol.wt.).

cause opaqueness. Besides this, ROS that penetrates across the aqueous humour from other tissue sites enhances the process.[15] Lipid peroxides ultimately resolve into malondialdehyde (MDA) as the end product.[30] It is possible that the carbamyl group of MDA could react with primary amino groups of protein and phospholipids of lenticular plasmalemmae by a crosslinking reaction forming schiff-base conjugate. Such a cross linking mechanism could initiate in the lipid bilayer of ocular cell membranes even by a small amount of MDA. Lipid peroxides can also oxidise SH groups of proteins by itself. The oxidation of the SH groups of proteins further undergo disulphide cross linking, forming high molecular weight aggregates leading to the maturation of cataract.

Biochemical studies shows that selenite+vitamin E treated non cataractous lens have a protein concentration of near normal value. Since vitamin E is capable of preventing oxidative damage, the selenite is probably incapable of exerting its oxidising effect on membrane SH groups. Burk et al[31] reported that selenium homeostasis is achieved by the regulation of urinary selenium excretion under physiological conditions. Hence the bulk of the administered selenium is metabolised and excreted when it is withdrawn. This would make the lens free from the oxidative insult of selenite.

The maintenance of a high concentration of glutathione (GSH) results in retention of soluble and insoluble protein at a normal concentration. Since vitamin E protects the SH groups from oxidation, the GSH concentration is maintained unaltered, as well as those of total sulfhydryl groups(TSH), Non-Protein sulfhydryl groups(NPSH) and Protein sulfhydryl groups(PSH). This demonstrates an unaltered biosynthesis and recycling of GSH as well as synthesis of proteins. A sufficient level of GSH is believed to be the main factor in the maintenance of the normal ascorbic acid concentration of the lens.[32] GSH and NADPH help maintain sufficient amount of ascorbic acid level, by recycling dehydroascorbate to ascorbate. Ascorbic acid (vitamin C), itself an effective antioxidant can also act synergistically with vitamin E. Vitamin C can rejuvenate vitamin E, and together can get against free radical damage in the lipids.[32] The maintenance of membrane integrity and transport lead to ion homeostasis. This is achieved by preventing the oxidation of critical SH groups of ATPases found in the lens membrane and also effectively scavenging the lipid peroxides and ROS generated by selenite. This is the most probable reason for minor changes in the ATPase activity and ion concentrations in the selenite + vitamin E treated crystalline lenses.

Histomorphological studies

Vitamin-E treated and control lenses showed an orderly arranged cellular architecture of the lens. Fibres in the innermost part of the lens are characterised by the presence of numerous ball and socket surface structures that cover the entire surface. These surface structures decrease in prominence with increasing distance from the centre of the lens. They are responsible for holding the adjacent fibres together, by fixing the ball-like surface structure of a fibre to the socket like surface structure of the adjacent fibres and vice versa. The fibres in and near the centre also have prominent interdigitations. The relatively small interdigitations of the superficial fibres allow movement of these fibres during accommodation. These interdigitations are responsible for holding together the mature fibres on one side of the sutural plane with those on the other.[33]

During selenite cataractogenesis the lenticular opacity began at the adult nuclear area, and slowly progressed to a pinhead opacity, enclosing the entire nuclear area. However, at this time the cortical area

is still clear. The age of fibres is a major factor in its susceptibility to selenite stress. The older cells of the foetal and embryonic nuclei are hardened, and are probably not as responsive to pathological change as the younger adult nuclear cells. This explains the initial morphological alterations in the adult nuclear area.

It is known that the rate of proliferation and differentiation of fibres in the rat lens is comparatively more rapid in the first few days following birth than in adult animals.[34] This causes the earlier differentiated foetal and embryonic nuclear fibre to have the normal fibre morphology. Since the selenite is administered, 10 days *post partum*, the lens fibre cells which are about to differentiate at the equatorial (bow) region are susceptible to the challenge of selenite. This is true since the initial changes were observed in the adult nuclear area by which time the embryonic and foetal nuclear area has already formed, and because of the fact that the movement of a single cell from the germinative region into the lens bow, with the final formation of a new non-nucleated lens fibre would take a few months.[35] Selenite cataract is easily produced within four to five days after injection of sodium selenite. The newly formed fibres push the deformed superficial fibre formed during selenite stress into the adult nuclear area where they progress to form a pinhead nuclear opacity. The process is accelerated due to the action of hydrolytic enzymes like acid phosphatase, and calpin. Both these enzymes are known to increase during selenite cataractogenesis.[15, 36]

Eventually it results in loss of fibre boundaries. The swollen fibres press against underlying and overlying fibres, which are propagated both inward and outward, possibily contributing to the damage observed within the inner nuclear and cortical layers of the lens. Besides, formation of vacuoles enhances lens fibre degradation.

The first damage in cataract induction is to the lens epithelium.[37,38] In our study epithelium was observed as a double-layered structure initially. This represents an abnormal nuclear migration secondary to selenite stress, which causes interruption in the orderly relationship between cell division and differentiation. As the cataract matured, the epithelial cells proliferated at an uncontrollable rate to form hyperplasia, and manifested into a mass of malformed cells or tissues.

Chemical injury is associated with hyperplasia of the lens epithelium.[39] Due to increased mitosis, the epithelial cells slide under each other forming plaques or they overshoot at the equator sending cells into the posterior subcapsular region. As the cataract matures, the oxidative process that initiated at the lens epithelial layer gradually extends into the cortical layer, leading to formation of high molecular weight aggregates, as observed by the increase in insoluble protein fraction.[40] This turns the lens opaque.

Electrophoretic studies

The important findings of the electrophoretic studies (Figures 10 and 11) were (1) the formation of insoluble protein (Table 2) and opacification of lens in the selenite-treated rats were associated with a decrease in several lens proteins, which on the basis of observed molecular weights, may correspond to cytoskeletal proteins and b-crystallins though this needs confirmation by use of suitable antibodies. (2) administration of vitamin E prevented the loss and insolubilisation of these proteins.

The crystallin subunits (a, b and g) have a molecular weight in the range of 18_32 kDa. The present study shows that the soluble proteins in these ranges decreased prominently in the advanced stages only. A major decrease or loss of protein band above 45 kDa among soluble proteins occurred from 3 days post selenite injection (Figures 10 and 11). Among insoluble proteins a loss of protein band was observed at 45 kDa. These high molecular weight protein bands represent cytoskeletal proteins. Matsushima[41] have reported loss of spectrin (235 kDa), actin (42 kDa), and 49 kDa and 70 kDa proteins in the nucleus of the selenite-treated Sprague-Dawley rat pups.

The major cytoskeletal components appear to participate in the development and maintenance of lens cell transparency[41] The vimentin _ actin -tubulin component is important in establishment of transparent cellular structure during lens cell differentiation in the cortex.[42-44]

Loss of protein bands at 45 kDa among soluble and insoluble fractions was observed by 3-5 days post selenite injection though major loss of 18_34 kDa proteins among soluble fraction was observed only in the advanced stages of selenite cataract (Figure 11). This suggests that the initial damage is to the cytoskeletal proteins rather than the crystallins during selenite cataract formation. The loss of cytoskeletal proteins contributes to the membrane damage and loss of fibre structure. Shearer et al[45] and Clark and Steele[46] reported that the inappropriate loss of cytoskeletal proteins within 72 hours after selenite injection corresponded to the abnormal variation in phase separation temperature Tc, a direct measure of the interactions between lens proteins.

During selenite cataractogenesis, the membrane critical sulfhydryl groups are oxidised, leading to the inactivation of Ca2+ ATPases or opening of ion channels in epithelial membranes. Calcium influx then causes activation of the calcium-dependent protease calpain II, which partially degrades a and b-crystallins. This leads to abnormal interaction of crystallins, in solubilisation of proteins and light scatter. Partially degraded b-crystallins become insoluble and scatters light.[47,48] Proteolyzed b-crystallin and non-proteolyzed g-crystallin polypeptides associate non-covalently to form light scattering urea soluble proteins.[49] The loss of proteins including crystallins following selenite injection in the present study could be due to the calcium activated protease calpain. *In vitro*, calpain is known to degrade purified lens cytoskeletal proteins like vimentin, actin and tubulin.[50,51] Velasco et al[52] have suggested that the dimeric and oligomeric b-crystallin products are due to intrinsic transglutaminase mediated crosslinking of proteins by formation of N^4 (g-glutamyl) lysine side chain bridges and the cytoskeletal proteins. They are susceptible to proteolytic action of enzyme calpain I and II. An increase in the SDS soluble(insoluble) protein fraction is due to the partial degradation, and aggregate formation of degraded crystallins. 14 kDa and 24 kDa crystallins mostly increased at different stages of selenite administration(Figure 10 and 11). Insolubilization and aggregation of crystallins and cytoskeletal proteins

can contribute to opacification.

In the present study, the protective effect of Vitamin-E on the formation of water-insoluble aggregates and prevention of opacification in the lenses of selenite-treated rats may correspond with the protection of proteins such as the crystallins and the cytoskeletal proteins. The biochemical mechanism for the prevention of degradation of cytoskeletal proteins, crystallins and opacification is due to the antioxidative property of vitamin E.

The selinite+vitamin-E cases has shown a 91.6% clear lens after the mega doses of vitamin E.[15] Vitamin-E is known for its free radical scavenging ability[53] and this helps to keep the membrane integrity of the epithelial

layer, and prevents further damage to the lens fibres. We also hypothesise that selenite and vitamin E could form some complexes in the blood or in the body fluids so that the former is prevented from entering into the lens and oxidizing the membrane proteins of lens epithelium. This prevents cataract formation.

It may not be a part of the medical dogma that swallowing pills of vitamin E or C every day keeps cataract away. However, there seems enough favourable evidence to evoke optimism.It is shown that both aging and certain diseases cause a decrease in the antioxidant level of the human body and this can initiate or enhance the progress of cataract.

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