ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Role of Topically-Applied Zinc Sulfate in Prevention of Sodium Selenite-Induced Cataract in Rabbits

***Baha'a A. Abdul-Hussein[1] ,Adeeb A. Alzubaidy[2]**

1. Lecturer in Pharmacology, Dept. of Pharmacology, College of Veterinary Medicine, University of Al - Qadissiya, Al-Qadissiya - Iraq
2. Assist. Prof. in Pharmacology, Dept. of Pharmacology, College of Medicine, Al-Nahrain University, Al-Kadhimiya-Baghdad- Iraq

Manuscript Info

Manuscript History:

Received: 11 January 2014
Final Accepted: 22 February 2014
Published Online: March 2014

Key words:
Cataract, Oxidative stress, Zinc sulfate

***Corresponding Author**

Baha'a A. Abdul-Hussein

Abstract

Background: cataract is the opacity of the lens which progressively impairs the light transmission to the retina and finally prevents the vision, this opacity results from the oxidative process in the eye. Zinc sulfate has an antioxidant property which can prevent the oxidative processes.
The present study aimed to evaluate the possible protective role of zinc sulfate eye drops against sodium selenite-induced cataract in rabbits.

Materials and Methods: In order to induce lens opacity, sodium selenite (0.01w/v) were injected intravitreal into eyes of the included rabbits which were already divided into control (distilled water) group (n= 6) and treatment (zinc sulfate) group (n= 6). Each of distilled water and zinc sulfate (tested drug) eye drops were instilled both prior and then along 21 days post injection of sodium selenite. By using ophthalmoscope grading criteria, the score of lens opacity (cataract maturity) was determined.

Results: In control group, the lenses, opacities could be induced within 48 - 72 hours of sodium selenite injection and their mean score reached to 4.0 (± 0.00). On the other hand and in comparison to that of control group, zinc sulfate eye drops could highly significantly retard the progression of the induced opacities and mean score of opacity was only 1.33 (± 0. 04) ($p < 0.01$).

Conclusions: Zinc sulfate eye drops exerted a detectable preventive effect against sodium selenite - induced cataract in rabbits.
.

Introduction

Cataract is the opacity of the lens which progressively impairs the light transmission to the retina and finally prevents the vision[1]. Oxidative stress on the lens represents the major factor that causes lens opacity. Oxidation of lens proteins SH- groups induce protein conformational changes leading to protein aggregation and opacification of the lens resulting in block of light transmission to the retina and then blindness[1,2,3,]. Zinc sulfate it is known with its antioxidant activity in vivo and in vitro and it was expected to prevent the oxidative process in the eye [4].

Aim of the study: The present study aimed to evaluate the possible protective role of zinc sulfate eye drops against sodium selenite – induced cataract in rabbits.

Materials and Methods

Animal and Housing: A group of 12 adult rabbits (Oryctologus cuniculus) aged about one year with a range of body weight of 1.5-2 kg were obtained from the animal house stock of the Department of Pharmacology, College of medicine, AL-Nahrain University, Baghdad-Iraq. Animals were kept on fresh trefoil diet, water at libitum, suitable temperature and normal light.

Groups of Study: The included rabbits were allocated into 2 groups, control (distilled water) (DW) group (n=6) and treatment (zinc sulfate) group (n=6). Right eye of each included rabbit was instilled with 2 drops 3 times / day of either distilled water (control group) or zinc sulfate (0.25%) (treatment group); such administration started five days prior to induction of cataract (i.e. prophylactic use) and continued thereafter for further 21 days after the cataract being induced (i.e. therapeutic use).

Preparation of sodium selenite solution: Amount of 10 mg of sodium selenite powder was dissolved in 100 ml distilled water to prepare the 0.01% w/v of selenite solution. A fresh solution was prepared for each use[5].

Induction of cataract: After the rabbits being anesthetized by intramuscular injection of 0.5 ml of ketamin (50 mg/ml), the induction of disease was done by inserting a needle 4 mm behind the limbus in sclera (measured by caliper) and 0.1 ml of sodium selenite solution (0.01% w/v) was injected intravitreally in the right eye [1,6]. After this single injection, the rabbits were monitored for caractogenesis which begun after one hour , tropicamide (0.5%) and phenylephrine (10%) were used to obtain maximum pupillary dilatation during examination, the rabbits were examined with slit-lamp in hour and thereafter in order to observe opacity progression [7]. After induction an attempt to detect the type of the induced cataract [2,3].

Preparation of zinc sulfate (0.25% w/v) eye drop (with pH 7.2±2**):** Zinc sulfate powder (250 mg) and 20 mg of benzalkonium chloride were dissolved in 100 ml of buffer solution (which was already consisted of 440 mg of phosphate powder in 100 ml distilled water) [5,8].

Ophthalmoscopic examination and opacity grading: The eye examinations were daily carried out in a dark room with a direct ophthalmoscopes and instillation of tropicamide (0.5%) and phenylephrine (10%) eye drops to obtain maximum pupil dilatation[9]. Opacities that obscured the red reflex were scrutinized from several angles of view to determine their location in relation to the lens. The grading of opacity included assessment of the area of clear red reflex from area without red reflex of retina[9]. By using ophthalmoscope grading criteria, the score of lens opacity (cataract maturity) was determined according to the classification of Mehra and Minassian, and Chylack et al.[10,11]. The ophthalmoscopic examination was done in dark room after measuring the pupillary response to light to avoid the influences of mydriatic drops. Slit-lamp (Topcon com.\ japan) was also used to evaluate the lens opacity [10,12,13]. At the end of experiment, the rabbits were killed and the lenses were extracted by posterior approach [14].

Then the lenses were sent directly to biochemistry lab. And a small part of the lenses was fixed in Gluteraldehyde (3%) for EM study [15, 16].

Each of reduced glutathione (GSH)[17] and malondialdehyde (MDA)[18] levels in these lenses were measured. Besides, electron microscope (EM) study was done for the lenses prepared for Semi- thin sections [15, 16.]

Statistical analysis:

All data were expressed as mean (±SD). Paired and unpaired t-tests were used accordingly for assessing the effectiveness of employed therapy for the right eyes of rabbits in a given group, to compare between the results of right and left eyes in the same rabbit, and the right eyes of rabbits of two groups. Chi-test was used whenever it was applicable (i.e. for independent qualitative data). $P<0.05$ was considered significant [19,20].

Results

According to opacity classification system [11,21], the type of induced cataract in the present study was found to be posterior sub-capsular one (PSC) (figure -1). Complete opacity (mature cataract) could be achieved in lenses of control group after 48-72 hours of intravitreal injection of sodium selenite. Figure (2) demonstrated the difference between two lenses: cataractous (after cataract being induced) one which appeared opaque and normal one which appeared transparent.

As shown in figure (3), cataract in the control group could advance with time to reach hypermature stage.

Control (DW) group: [**Figure (4) & its associated table]**

Prior the cataract induction, lenses of right eyes of the included rabbits were intact, transparent, and had intact response to light; instillation of DW eye drops for 5 days did not affect them and the mean score of opacity remained 0.0 (±0.0). After cataract being induced and instillation of DW eye drops was continued, the mean score of opacity increased to be 4.0 (± 0.00); compared to pre induction value, such increment was significant. These eyes lacked their response to light and persisted so along the trial period.

Zinc sulfate (0.25%) group: [Figure (5) & its associated table]

Prior the induction of cataract the lenses of right eyes of the included rabbits were intact and transparent and the mean score of cataract maturity was (0 ± 0.00) t both pre and post instillation of zinc sulfate eye drops for 5 days. After cataract being induced and instillation of zinc sulfate was continued, the mean score of cataract maturity was found to be (0.8 ± 0.01), (1.2 ± 0.02), and (1.33 ± 0.04) at 7th , 14th, and 21st day respectively; comparing to pre induction value, there were significant differences.

Compared to effects of DW eye drops, zinc sulfate (0.25%) eye drops appeared more efficient in cataract prevention along the trial period (Table-1).

Regarding response to light, zinc sulfate (0.25%) eye drops did not affect any of included eyes along its use for 5 days prior cataract induction. After cataract being induced in the 6 included right eyes, response to light was lost in 2 eyes at day 7th and then in only one eye at days 14th and 21st. There was significant difference when being compared to those of distilled water group.

EM study:

Control (DW) group: As shown in Figure (6), oxidative and sclerotic effect of selenite on the lens proteins rendered the cytoplasm of lens to loss its featureless, homogenous and dens appearance. As a cause of the lenticular opacity, there were thick darkly stained aggregations (insoluble proteins) inside the enlarged irregularly shaped fiber making a connected network across lens fibers. These aggregations are surrounded by clear or lighter areas which probably resulted from losing of homogenous appearance of the cytoplasm.

Treatment (Zinc sulfate 0.25%) group: Zinc sulfate could prevent the aggregation of the proteins and the cytoplasm seemed approximately homogenous and normal (Figure-7).

MDA and GSH levels:

Table (2) demonstrated the MDA and GSH levels that measured at the end of prophylaxis i.e., after 5 days of instillation of either DW or zinc sulfate 0.25% eye drops, whereas table (3) expressed the significance of the obtained differences in these levels among included groups.

(A) (B)

Figure (1): Slit- lamp photographs for rabbit eyes; A: Left normal eye. B: Right cataractous eye (with posterior subcapsular opacity).

Figure (2): Normal (transparent) and Cataractous (opaque) Lenses of Rabbit

----

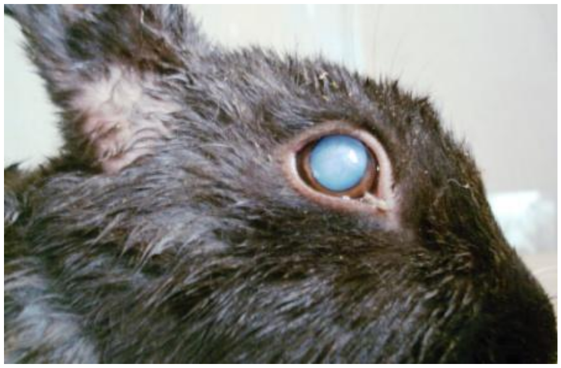
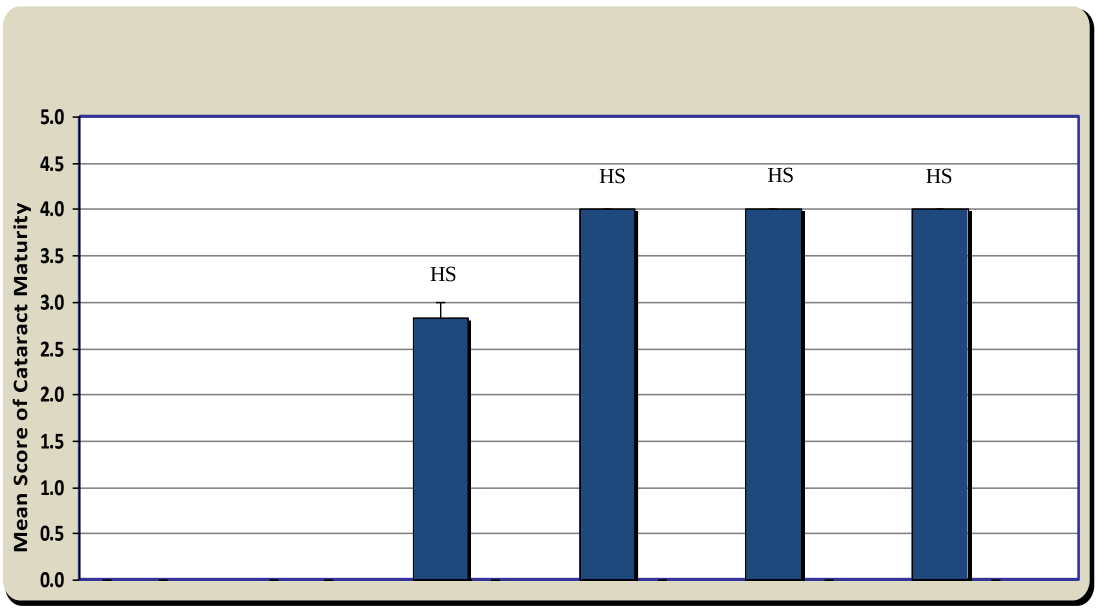


Figure (3): Hypermature Induced Cataract in Lens of Rabbit of Control (Distilled Water) group

----

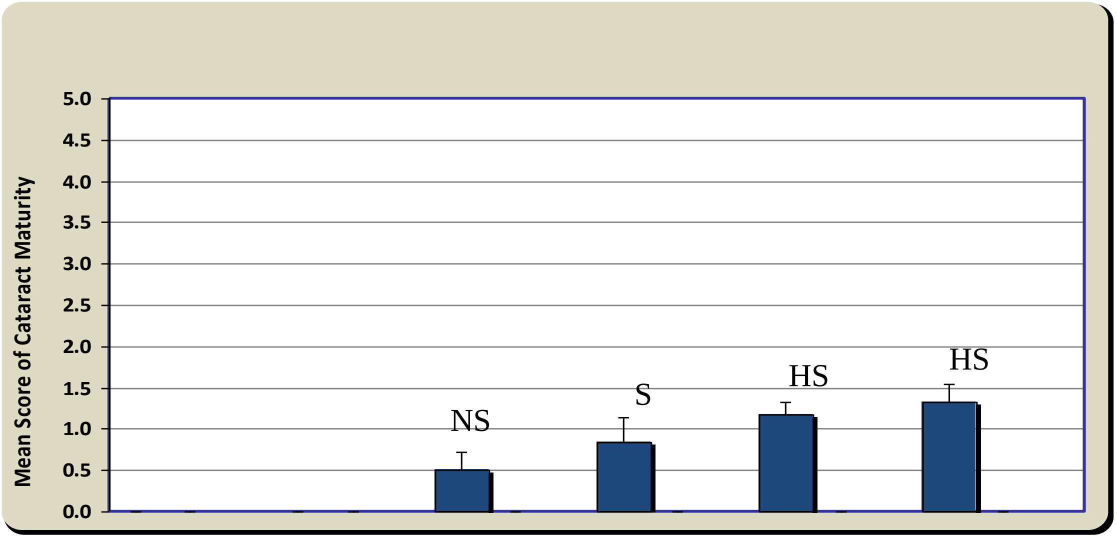


	Pre-Induction (Days)		Post-Induction (Days)			
	0	5	1	7	14	21
MSCM*	0.0	0.0	2.8	4.0	4.0	4.00
±SEM	0.00	0.00	0.17	0.00	0.00	0.00

MSCM*= Mean Score of Cataract Maturity; SEM = Standard Error of Mean; HS= high significant difference (p < 0.01) compared to corresponding 0 time value

Figure (4) & Associated Table: Effect of DW eye drops on Mean Score of Cataract Maturity in Lenses of Right Eyes of Six Rabbits during Pre- and Post-Induction of Cataract

----



	Pre-Induction (Days)			Post-Induction (Days)			
	0	5		1	7	14	21
MSCM*	0.0	0.0		0.5	0.8	1.2	1.33
±SEM	0.00	0.00		0.02	0.01	0.02	0.04

MSCM*= Mean Score of Cataract Maturity; SEM = Standard Error of Mean; =0.25 mg/drop, NS= No Significant difference ($p > 0.05$) compared to corresponding 0 time value, S = Significant difference ($0.01 \leq p < 0.05$) compared to corresponding 0 time value, HS= high significant difference ($p < 0.01$) compared to corresponding 0 time value**

Figure (5) & Associated Table: Effect of Zinc sulfate eye dropon Mean Score of Cataract Maturity in Lenses of Right Eyes of Six Rabbits during Pre- and Post-Induction of Cataract**

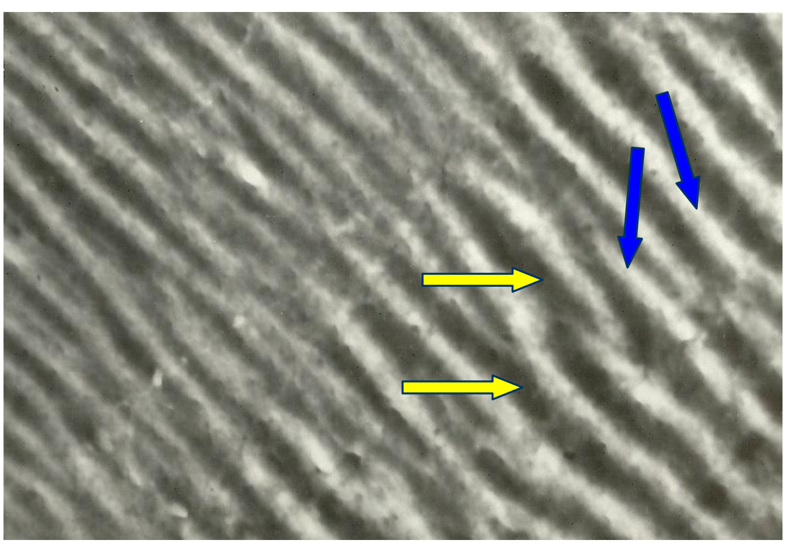


Figure (6): Electron micrograph; Longitudinal section of the cataractous (control) lens shown as darkly stained aggregations (yellow arrows) which surrounded by the clear areas (blue arrows) in the fibers and losing the homogenous state of cytoplasm of the cataractous lens

fibers. (13500 x)

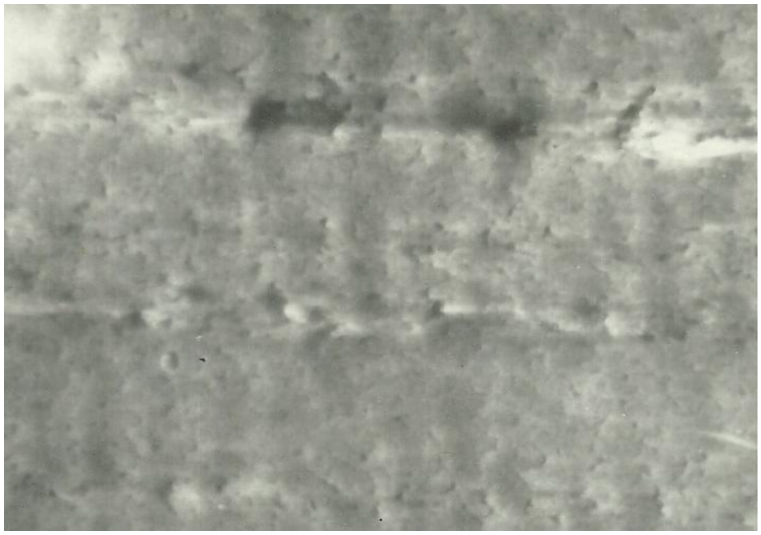


Figure (7): Electron micrograph; Longitudinal section of the lens protected with zinc sulfate (0.25%) eye drop, shown as homogenous cytoplasm. (13500 x)

Table (1): Significance of differences between zinc sulfate (0.25%) group compared to distilled water group regarding the mean score of cataract maturity of right eyes of rabbits.

	Pre-induction (Days)		Post-induction (Days)		
Group	**0**	**5**	**7**	**14**	**21**
Distilled water	NS	NS	HS (Z)	HS (Z)	HS (Z)

0 = Baseline (Pre-treatment), NS= No Significant difference ($p > 0.05$) , HS = Highly significant difference ($P \leq 0.01$), (Z) = the lowest value belongs to zinc sulfate group.

Table (2): Levels of MDA* and GSH measured at the end of prophylactic instillation of DW or zinc sulfate (0.25%) eye drops.**

	Mean (±SD) (µMol/L)	
Group	**MDA**	**GSH**
Normal eyes #	0.034 (± 0.042 X10^{-2})	0.0013 (±0.17 X10^{-5})
DW	0.21 (±0.25 X10^{-2})	0.0005 (±0.5 X10^{-5})
Zinc sulfate (0.25%)	0.036 (±0.27 X10^{-2})	0.0012 (±0.7 X10^{-5})

***= malondialdehyde, **= reduced glutathione , # = 0-time (i.e., prior cataract-induction and received no treatment)**

Table (3): Significance of differences in MDA* and GSH levels among included groups**

Group	Normal eyes #	DW	Zinc sulfate
Normal eyes #		HS (0)	NS
DW	HS (0)		HS (Z)
Zinc sulfate	NS	HS (Z)	

***=malondialdehyde,**= reduced glutathione, # = 0-time (i.e., prior cataract-induction and received no treatment), HS= high significant difference (p < 0.01), NS= Not Significant difference (p > 0.05), 0 = the better result (i.e., highest MDA level or lowest GSH level) belongs to 0-time group, Z= the better result (i.e., highest MDA level or lowest GSH level) belongs to zinc sulfate group**

Discussion

Cataract is progressively impairs the light transmission to the retina and finally prevents the vision [22], therefore, it seems so important if a topically-applied drug can be proved to be effective whether prophylactically (i.e., to prevent the development of cataract in a susceptible patient) and/or therapeutically.

In the present study, zinc sulfate (0.25% w/v) eye drops instilled 3 times/day for 5 days did not affect the lens transparency in normal eyes. Besides, it was able to prevent selenite to raise the mean score of cataract maturity to its expected value that was obtained in the DW group. Furthermore, when being continued for further 21 days, zinc sulfate (0.25% w/v) eye drops instilled 3 times/day could prevent cataractogenesis in better manner (P < 0.01) than DW did.

The EM study showed that zinc sulfate protected the lens proteins from opacification probably by prevention of the proteins aggregation and thus cytoplasm seemed approximately homogenous.

Hence, results of the present study pointed out to the beneficial prophylactic anti-cataract effect of zinc sulfate (0.25% w/v) eye drops.

Ito et al[23], and Chung et al[24] suggest that nitric oxide (NO) and H_2O_2 had an important role in the development of selenite-induced cataracts. Other studies found that minerals, including zinc, are co-factors for naturally occurring antioxidant enzymes [25,26,27] , and the plasma levels of zinc were found to be significantly low in cataractous patients [27,28]. Zinc when found in sufficient quantities will increase catalytic activity of zinc – superoxide dismutase which is an important enzyme that protect the lens against cataract [29] ; this appeared in accordance with what was found in the present study regarding results of MDA and GSH levels determined in lenses that received zinc sulfate which pointed out the antioxidant activity of zinc sulfate which probably takes place in the protection the lens proteins from selenite induced cataractogenesis. A great challenge expected between inducing agent (selenite) which injected intravitreal and was expected to cause opacity within 48 hours and zinc sulfate that instilled topically, which was expected to expose to barriers in the eye to reach the lens [1,30,31]. In addition to that, the most insoluble proteins are found in the lens nucleus, whereas most soluble proteins are in the cortex [32]. One of the most important sign of lenticular opacity is deterioration of response to light [12,13]. The resulting opacity, however, needs to be located at or near the visual axis for it to affect vision and become clinically important [8,20,21,32]. In the present study, when the tested drug (zinc sulfate) had been used prophylactically, light response could be kept intact; this probably as a result of capability of zinc sulfate to prevent the extension of cataractogenesis to the nucleus of lens and thus the light could pass through the lens to retina.

Conclusions

Zinc sulfate (0.25% w/v) eye drops instilled with 2 drops 3 times / day exerted a detectable preventive effect against sodium selenite - induced cataract in rabbits; it seemed to be apparently safe and tolerable therapy along the trial period in the present study.

Recommendations

Controlled clinical studies are warranted in order to evaluate the prophylactic role of zinc sulfate eye drops in patients with high risk to develop cataract.

Acknowledgment

We grateful to the ophthalmologist Dr. Hassanain A. AL-Saedy, and the ophthalmologic consultant in Al-Dewaniyah Teaching Hospital, for their kind assistance in establishment of the present work.

References

1- Cotlier E. (1995): Physiology of the lens. In: Moses R.A. and Hart W.M. Alder's Physiology of the eye Clinical Application. 9th ed. Mosby Company. St. Louis. Pp.268-290.

2- Harper RA. and Shock JP. (2007): Lens: In: Riordan-Eva P. and Whitcher PJ. : Vaughan & Asbury's General Ophthalmology. 16th. ed. McGraw-Hill Companies. Boston. Chapter 8.

3- Biju PG., Rooban BN., Lija Y., Devi VG., Sahasranamam V. and Annie Abraham. (2007): Drevogenin D prevents selenite-induced oxidative stress and calpain activation in cultured rat lens. Molecular Vision . 13:1121-1129.

4- Powell S.R. (2000): The Antioxidant Properties of Zinc. 130 , 5: Pp. 1447-1454. The journal of nutrition.

5- British Pharmacopoeia. London, Her Masjesty's Stationary office (2004): Sodium selenite , zinc sulphate. Vol. I &II.

6- Kanski J. (2007): Kanski clinical ophthalmology A systemic approach. 6th ed. Lippincott's. Philadelphia and Sant louis. Pp. 355,357.

7- Samuel J., Ziegler JR. and Datlies MB.(2003): Pathogenesis of Cataracts. In: Thomas M., Aaberg SR., Richard L., Mark B. and Dharam V. Duane's Foundations of Clinical Ophthalmology. Lippincott Williams & Wilkins. Philadelphia. Chapter 72b.

8- Allen L.V., Popovich N.G. and Ansel H.C. (2005): Ansel's Pharmaceutical Dossage Forms and Drug Delivery Systems. 8th ed. Lippincott Williams and Wilkins. Philadelphia. Pp. 540-569.

9- Macdonald M. (2000): The examination of the eye. In: Munro J. and Edwards C. Macleod's Clinical Examination. 10th .ed. Churchill Livingstone. Edinburgh. Pp. 257-271

10- Mehra V. and Minassian DC. (1988): A rapid method of grading cataract in epidemiological studies and eye surveys. British Journal of Ophthalmology. 72; 801-803.

11- Chylack LT., Wolfe JK., Singer DM., Leske MC., Bullimore MA., Bailey IL., Friend J., McCarthy D., and Wu SY. (1993): The Lens Opacities Classification System III. The Longitudinal Study of Cataract Study Group. Arch Ophthalmol. 111:831-836.

12- Ahuja M. (2003): Ophthalmology Handbook. 1st ed. India Binding House. Delhi. Pp.164-182.

13- Jaffe NS., Jaffe MS. and Jaffe GF. (1990): Cataract surgery and its complications.5th ed. Mosby. ST. Louis. Pp.6-18

14- Chitra V., Lakshamib KS., Sharmaa S., Patidara A. and Rajeshb T. (2009): lisinopril attenuates selenite induced experimental cataract in vitro study . International journal of pharmacy and pharmaceutical sciences. 1: 17-23.

15- Russell N J., Royland J E., McCowley E L. and Shearerj T R.(1984): Ultrastructural Study of Selenite-lnduced Nuclear Cataracts. Invest Ophthalmol Vis Sci. 25:751-757.

16- Hayat, M.A (1986): Basic techniques for transmission electron microscopy. Acad.press Inc.Harcourt Brace Jovanovich. Sandiego, New York, Berkeley, Boston, London, Sydney, Tokys Toronto, Pp:226-231.

17- Moron MS., Depierre JW., and Mannervik B. (1979): Levels of glutathione, glutathionereductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta. 82:67.

18- Buege JA., and Aust SD. (1978): microsomal lipid peroxidation. Meth. Enzymol. 51; 302-310

19- Daneil W.W. (1983): Biostatistics: A foundation for analysis in the health sciences. 3rd rd. John Wiley and Sons. New York. Pp. 89-92, 102-103.

20- Hill A.B. (1991): Brodford Hill's Principles of Medical Statistics. 12th ed. Hodder and Stoughton. London. Pp. 78-84.

21- Datiles MB. and Magno BV. (2003): Cataract: Clinical Types. In Thomas M., Aaberg SR., Richard L., Mark B. and Dharam V. Duane's Foundations of Clinical Ophthalmology. Lippincott Williams & Wilkins. Philadelphia. Chapter 73.

22- Stifter E., Sacu S., Benesch T., and Weghaupt H**.** (2005): Impairment of Visual Acuity and Reading Performance and the Relationship with Cataract Type and Density. Investigative Ophthalmology and Visual Science. 46:2071-2075.

23- Ito Y., Nabekura T., Takeda M., Nakao M., Terao M., Hori R. and Tomohiro M.(2001): Nitric oxide participates in cataract development in selenite-treated rats. Curr Eye Res. 22: 215-220.

24- Chung L K., Ching KC**.,** Hsu WM. (2002): Nitric oxide levels in the aqueous humor in cataract patients. Journal of cataract and refractive surgery. 28: 507-512

25- National Eye Institute (2001). www.nei.nih.gov.

26- American academy of ophthalmology (2007) .Eye Care America. Antioxidant Vitamin and Mineral Supplements and Cataract Prevention and progression

27- Gupta SK., Selvan VK., Agrawal SS. and Saxena RS. (2009): Advances in pharmacological strategies for the prevention of cataract development. Eye journal. 23:10-16.

28- Bhat KS. (1987): Nutritional status of thiamine, riboflavin and pyridoxine in cataract patients. Nutr Rep Inter;36:685-92

29- Olofsson EM, Marklund SL, Karlsson K, Brännström T, Behndig A. (2005): In vitro glucose-induced cataract in copper-zinc superoxide dismutase null mice. Exp Eye Res. 81:639-46.

30- Duancan G. and Wormston M. (2003): Physiology of the Lens. In Thomas M., Aaberg SR., Richard L., Mark B. and Dharam V. Duane's Foundations of Clinical Ophthalmology. Lippincott Williams & Wilkins. Philadelphia.

31- Moroi SE. and Lichter PR. (2006): Ocular Pharmacology. In Goodman and Gillman's The Pharmacological Basis of Therapeutics. 9th ed. McGraw. Hill. New York. Pp. 1619-1644.

32- Kardong K. (2008): Vertebrates: Comparative anatomy, function, evolution.5th ed. Boston. McGraw-Hill. Pp.676-677.