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Alpha Lipoic Acid: A Potent Antioxidant in Treatment of Cataract

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ABSTRACT

Opacity within the clear natural crystalline lens of eye, which gradually results in vision deterioration, is called as cataract. Causes of cataract formation are ageing, free radical generation, diabetes, denaturation protein due to oxidative stress. Antioxidants protect the eyes by reducing free radical damage. The existing study evaluated the in-vitro anti-cataract activity of α- lipoic acid against glucose induced caractogenesis using goat lenses. Clear isolated goat lenses are incubated in the manufactured aqueous humor and divided into six experimental groups. The α-lipoic acid at a dose of 15µg/ml, 30µg/ml, and 60µg/ml is incubated simultaneously with glucose (55mM) and glucose (5.5mM) for a span of 24 hrs. Ascorbic acid (40 µg /ml) is used as the standard drug. At the end of the incubation lens opacity is measured by photographic evaluation. The lipoic acid shows significant prevention of cataractogenesis of eye lenses by alpha lipoic acid at 60µg/ml.

Keywords: Cataract, Ascorbic acid, α- lipoic acid, Antioxidant.

INTRODUCTION

Lens transparency affect mainly due to optical dysfunction of crystalline lens. It decreases the extent of incoming light and results in deterioration of the vision. Relatively 25% of the population of 65% over 80 has serious loss of vision because of cataract. Cataract progress is usually a very slow or gradual process but in some cases it could occur fast and it generally affects both eyes. Senile cataract, also known as age related cataract, is the most commonest type of cataract affect uniformly persons of either sex usually above the age of 50 years. It is one of the most important leading causes of blindness and visual defect worldwide covering around 42% of overall blindness thus, increasing load to health care system.

The hallmark symptom of cataract is decreased vision clarity. Changes in refractive error may also occur. Mild cataracts that do not significantly affect vision may be identified clinically. In such cases, patients may be consult to watch for vision changes, such as reduced visual acuity or contrast sensitivity or seeing images, which may be constant or occur only under certain conditions. The patient should be advised that the presence of lenses opacity does not necessarily warrant surgical interventions. When vision loss affects the ability to perform activities of daily life, consideration should be given cataract extraction.

Various aspect such as daylight, UV Radiation, diabetes, dehydration, oxidation of lens protein and peroxidation of lipid, hyperglycemia attribute to the generation of lens opacities in the older individual. It is also caused due to the harm to the long lived lens protein which as a result of oxidation due to the formation of oxidative radicals, various risk factors such as nutritional deficiency, sunlight, environmental factors, lack of consumption of antioxidants and diabetes can also increases the risk of

cataract. According to the animal study it was found that diabetes one of the leading risk factor involved in progress of cataract. During hyperglycemia extra cellular glucose diffuses into the lens, which can lead to post-translational alteration. The cataractogenesis is mainly due to the synthesis and aggregation of extra sorbitol in the lens fibers and subsequent osmotic stress are mainly responsible factors. Sorbitol is synthesized by aldose reductase utilizing NADPH and does not easily cross cell membranes; it can accumulate in cells and cause harm by distributing osmotic homeostasis. Pathophysiological mechanism behind the formation of cataract is deficient glutathione (GSH) levels, which involve keeping the lens protein in their reduced form. However, in the cataract GSH level were found to be significantly reduced when compared to normal. Oxidative stress is another mechanism involve in the cataract development, which causes oxidation of lens protein and affect the lens. Reduce concentration of glutathione, ascorbate and antioxidant enzyme such as catalase, superoxide dismutase, glutathione reductase and glutathione peroxidase which increasing age in the human eye were the main factors involving in the generation of cataract.

Oxidative stress:

The paradox of life fortunately, the oxygen in the air around us reacts sluggishly with most other compound. However, the addition of extra, unpaired electron (reduction) to the oxygen molecule generates the superoxide radical (O_2^-) which weakens the oxygen- oxygen bond rendering it slightly more reactive. Species that contain one or more unpaired electron and are capable of independent existence are known as free radical. The reactivity of free radicals varies widely and some none radical oxygen derived compounds are in fact more reactive than the oxygen free radicals. The collective term, reactive oxygen species (ROS) is there for usually instead use as it includes both the oxygen free radical and non radical derivatives of O_2.

MATERIAL AND METHODS

Requirements

Test drug: Alpha Lipoic Acid

Chemicals:

Sodium chloride (NaCl), Potassium chloride (KCl), Magnesium chloride ($MgCl_2$), Sodium bicarbonate ($NaHCO_3$), Calcium Chloride ($CaCl_2$), Glucose, Penicillin, Streptomycin , Ascorbic acid.

Instruments: Incubator, Wired mesh, Petri dish.

Dose selection: [1] α-lipoic acid–15, 30 and 60µg/ml.

Standard Ascorbic Acid: 40µg/ml.

Collection of eyeballs:

Goat eye balls were used in the present study. They were obtained from the slaughterhouse instantly after slaughter and transported to laboratory at 0-4^o C. [2]

Procedure:

Lens Culture:

A Fresh goat eyeballs were obtained from the slaughter house and instantly transported to the laboratory at 0-4°C. The lens were detached by extra capsular extraction and incubated in unreal aqueous humor (NaCl 140 mM, KCl 5mM, $MgCl_2$ 2 mM,$NaHCO_3$ 0.5 mM, $NaHPO_4$ 0.5 mM, $CaCl_2$ 0.4 mM and glucose 5.5 mM) at room temperature and maintain pH 7.8 by addition of $NaHCO_3$). Penicillin G 32% and streptomycin 250 mg% added to the culture media to inhibit bacterial contamination. At high concentration, glucose the lens was metabolized through sorbitol pathway and accumulation of polyols causing over hydration and oxidative stress. This leads to cataractogenesis.

Induction of in-vitro cataract:

Glucose at a concentration of 55 mM was used to induce cataracts. At high concentrations, glucose in the lens metabolizes through the sorbitol pathway. Accumulation of polyols (sugar alcohols) causes over hydration and oxidative stress. This generates cataractogenesis. These lens

were incubated in artificial aqueous humor with different concentration of glucose (5.5 mM) served as normal control and 55 mM serve as toxic control) for 72hours.

Study Design and Groups:

Goat lenses were divided into six groups of six lens each and incubated as follows:

Group I: Aqueous humor + Glucose 5.5 mM (Normal control).

Group II: Aqueous humor + Glucose 55 mM (Negative control)

Group III: Aqueous humor + Glucose 55mM + 40µg/ml Ascorbic acid. (Standard)

Group IV: Aqueous humor + Glucose 55mM + 15µg/ml alpha lipoic acid (Test I)

Group V: Aqueous humor + Glucose 55mM + 30µg/ml alpha lipoic acid (Test II)

Group VI: Aqueous humor + Glucose 55 mM + 60 µg/ml alpha lipoic acid (Test III)

Photographic Evaluation:

Lenses were placed on a wired mesh with the posterior surface touching the mesh, the pattern of mesh number of squares clearly visible through the lens was observed to measure lens opacity.

The degree of opacity was graded as follows:

"0": absence of opacity.

"1": slight degree of opacity.

"2": presence of diffuse opacity.

"3": presence of extensive thick opacity

Preparation of lens homogenate

After 72 hours of incubation, homogenate of lenses was prepared in tris buffer (0.23 M, pH- 7.8) containing 0.25 × 10-3 M EDTA and homogenate was adjusted to 10% w/v which was centrifuged at 10,000 G at 4ºC for 1hour and the supernatant was used for the estimation of biochemical parameters.

Biochemical parameter: [3]

Estimation of total protein content:

To 0.1 ml of lens homogenate, 4.0ml of alkaline copper solution was added and allowed to stand for 10min. Then, 0.4 ml of phenol reagent was added very rapidly and mixed quickly and incubated in room temperature for 30 mins for color development. Reading was taken against blank prepared with distilled water at 610 nm in UV-visible spectrophotometer. The protein content was calculated from standard curve prepared with bovin serum albumin and expressed as µg/mg lens tissue.

Statistical Analysis: [4]

All data were expressed as mean ± SD. All data were analyzed with SPSS/10 student software. Hypothesis testing methods include done way analysis of variance (ANOVA) followed by LSD. The values are expressed as mean ± S.D. and results were considered significantly different if $P<0.05$. Statistical variations are compared as follows:

Normal Goat lens vs Goat lens + Glucose 55mM, Goat lens + Glucose55mM vs Goat lens + Glucose55mM + alpha lipoic acid.

RESULT AND DISSCUSSION

In- vitro anti-cataract activity:

After 8 hr of incubation lenses with glucose 55mM shows opacification at periphery, on the posterior surface of the lens. At the end of 72 hrs complete opacification is progressively increased to words the center.

Photographic evaluation:

After 72 hours of incubation, transparency was maintained in the Group I (normal control group) [fig. 1] but there was complete loss of transparency in the Group II (negative control group) [fig. 2] indicating complete cataractogenesis. Group III (positive control group) [fig. 3] containing lens treated with standard ascorbic acid were squares of the graph paper were visible through the lenses. Goat lenses of groups containing escalated doses of the alpha lipoic acid (Group IV, V) were less hazy and the squares of the graph paper were visible through the lenses indicating suppression of cataract formation [fig. 3, 4, 5)]. Group VI (containing 60µg/ml) was more effective in suppressing cataract formation [fig.5] than Group IV [fig. 4] and Group V [fig. 3].

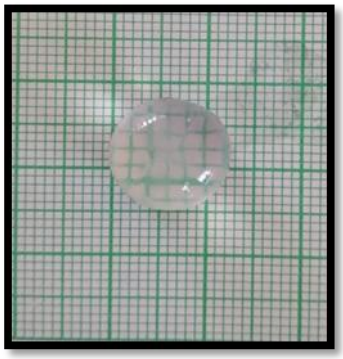


Fig. 1 (Normal group)

fig. 2 (Negative group)

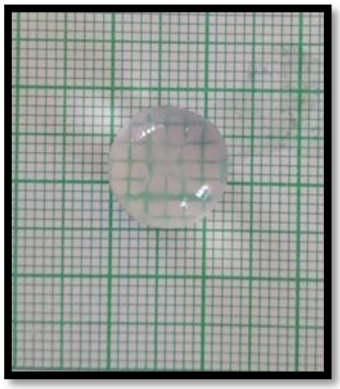


Fig. 3 (Positive control froup)

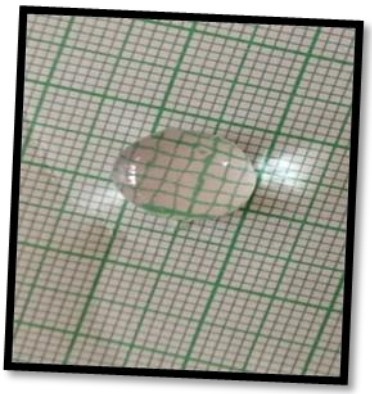


fig. 4 (Positive control group)

fig. 5 (Positive control group)

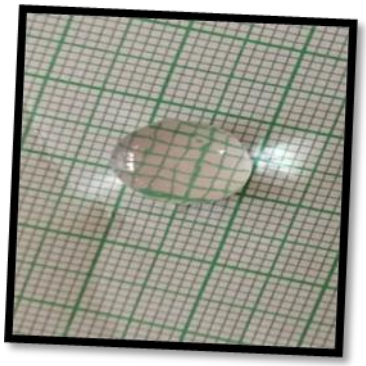


fig. 6 (Standard group)

Table No.1: Effect of alpha lipoic acid on degree of opacity on lens by glucose induced cataract:

Sr. No	Compound	Degree of opacity
1	Normal	0
2	Negative control (Glucose 55 mM)	3
3	Positive control (Ascorbic acid 40µg/ml)	1
4	Test I(Alpha lipoic acid 15µg/ml)	2
5	Test II (Alpha lipoic acid 30µg/ml)	1
6	Test III (Alpha lipoic acid 60µg/ml)	0

Normal control - Zero degree opacity occurred, clear lens is obtained.

Negative control - Presence of extensive thick opacity, because of high conc. Of glucose induced cataractogenesis.

Positive control (Ascorbic acid 40µg/ml) – lenses show slight degree of opacity, clear lens was not found.

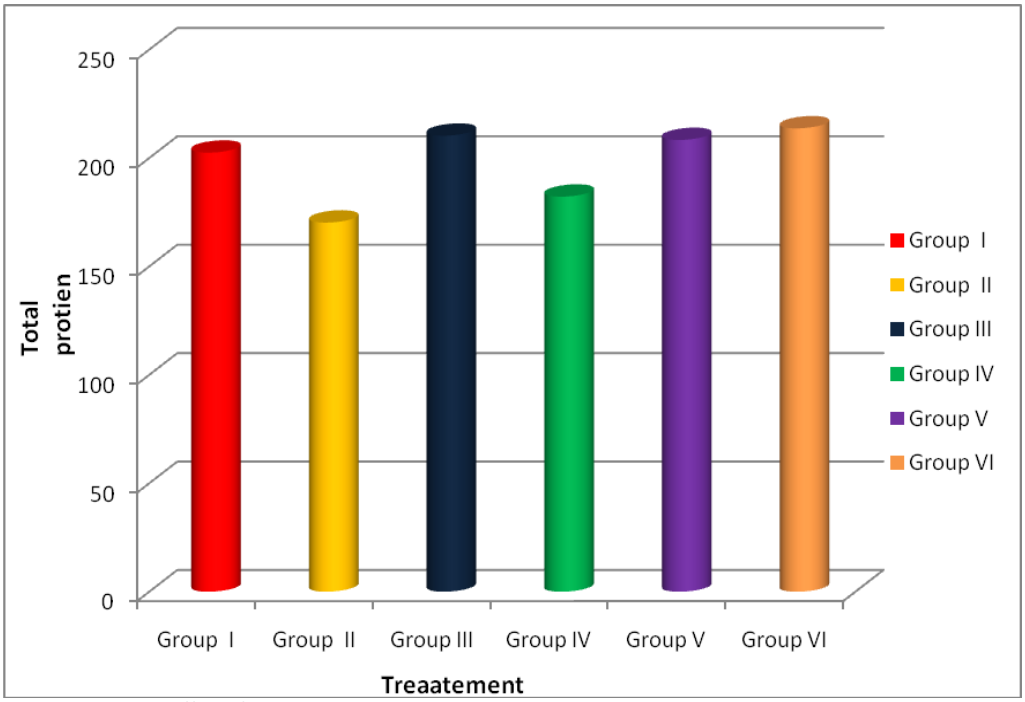
Test I (alpha lipoic acid 15µg/ml) – lenses show slight degree of opacity, clear lens was not found.

Test II (alpha lipoic acid 30µg/ml) – lenses show moderate degree of opacity, clear lens was not found.

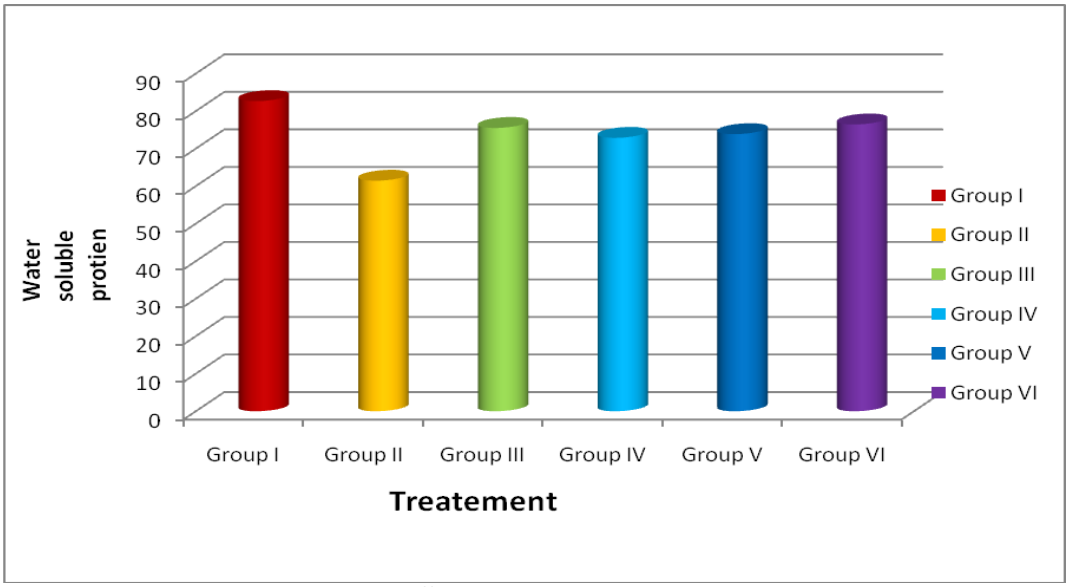
Test III (alpha lipoic acid 60µg/ml) – Zero degree opacity is occurred, clear lens is obtained. Test drug inhibits cataractogenesis.

Table No.2: Effect of Alpha lipoic acid on Protein levels (total proteins and water soluble proteins) in Goat lens homogenate after 72 hours of incubation in glucose 55 mM induced cataract:

Group No.	Treatment	Total Protein(mg/gm)	Water Soluble Protein(mg/gm)
I	Normal (Glucose 5.5mM)	202.50± 2.239	82.662± 3.041
II	Negative control (Glucose 55mM)	170.25± 2.095##	61.443± 3.055##
III	Standard (Glucose 55mM+Ascorbic Acid 40µg/Ml)	210.31± 2.649**	75.512±2.148**
IV	Test I(Glucose 55mM+ALA 50 mg/ml)	182.20± 2.139	72.866± 1.280*
V	Test II (Glucose 55mM+ALA 100 mg/ml)	208.34± 1.648**	73.859± 1.655**
VI	Test III (Glucose 55mM+ALA 200 mg/ml)	213.70± 1.687**	76.444± 1.150**



Graph No. 1 Effect of alpha lipoic acid on Total protein levels in Goat lens in glucose induced cataract



Graph no: 02: alpha lipoic acid effect on water soluble protein contained in goat eye lenses.

N=6, values are expressed as Mean ± SEM. Comparison were made as follows, # p < 0.05, ## p< 0.01 when compared with normal control.* p <0.05, ** p < 0.01 when compared with negative control. (Values are compared on 72hr by one way ANOVA Dunnett t test) (N.S) Non Significant.

The treated lenses (Group-II) with glucose 55 mM solution showed significantly low concentrations of proteins (total and water soluble proteins) in the lens homogenate ($P<0.01$) compared with normal lenses (Group-I). Ascorbic acid treated lenses (Group-III) and Lenses treated with alpha lipoic acid (Group-IV, V, VI) showed higher concentrations of proteins (total and water soluble proteins) ($P<0.01$) compared with Glucose 55 mM treated lenses (Group-II).

DISCUSSION

Anti-cataract activity:

The parameters which are considered commonly in cataractogenesis are electrolytes sodium and potassium malondialdehyde (MDA) and proteins (total proteins including water soluble proteins). Incubation of goat lens in media containing 55 mM glucose concentration had shown depletion in total protein content in general and water soluble protein in particular in the lens homogenate. Alteration of Na^+/K^+ ratio due to reduction in Na^+/K^+ ATPase activity in the lens causes Hydration and inflammation of the lens and alteration of the protein content. Both of these are responsible for formation of cataract. This study showed elevated total and water soluble proteins in ascorbic acid and alpha lipoic acid treated groups. Therefore Ascorbic acid and alpha lipoic acid treated groups seem to prevent pathogenesis of cataract formation. This may be due to their free radical scavenging activity.

In Normal control, after 72 hr. of incubation of lens in aqueous humor and 55 mM of glucose the lens was clear because of low conc. of glucose that doesn't show any effect on lens and numbers of squares are visibly seen through lens. The lens showed zero degree of opacity.

In Negative control, lens was incubated for 72 hrs in aqueous humor and 55 mM glucose solution for induction of high conc. of glucose in lens which gets metabolized through sorbitol pathway and accumulation of polyols causing hydration and oxidative stress. This leads to cataractogenesis. The lens showed extensive thick opacity.

In Standard group, after 72 hr. of incubated lens in Aqueous humor + 55 mM Glucose+ 40µg/ml Ascorbic acid std. drug, numbers of squares were not visibly seen through lens was compared with Test-3, the lens showed minor degree of opacity.

In Test- 1 & Test -2, after 72 hr. of incubation of lens in Aqueous humor + 55 mM Glucose + 15 µg/ml and 30µg/ml Alpha lipoic acid test drug, number of squares were not clearly visible through lens as compared to Alpha lipoic acid 60 µg/ml test-3 drug, the lens show slight degree of opacity.

In Test- 3, after 72 hr. of incubated lens in Aqueous humor + 55 mM Glucose + 60 µg/ml alpha lipoic acid drug, no. of squares was visibly seen through the lens. The lens showed absence of opacity, because the test drug inhibits cataractogenesis and oxidative stress.

In the in-vitro model for inducing cataract using 55 mM glucose solution provide an efficient model on isolated goat lens. Incubation of goat lenses in the media containing elevated glucose (55 mM) concentration has provoke cataract and has shown to cause considerable drop in Na^+/K^+- ATPase activity, with progression of opacity. The impairment of Na^+/K^+- ATPase causes accumulation of sodium and loss of potassium with hydration and inflammation of the lens fibers leading to cataractogenesis. This variation in the Na^+, K^+ ratio changes the protein content of the lens, leading to a reduce in total proteins causing lens opacification. The imbalance of Na^+ and K^+ was inhibited due to an action of alpha lipoic acid which corrects imbalances in the polyol pathway by decreasing aldose reductase activity, sorbitol concentration, and intracellular glucose. This effect can be certified to adoptogenic potential of Alpha lipoic acid.

Glucose induced cataract on goat eye was evaluated against alpha lipoic acid. Alpha lipoic acid significantly protected the lens morphology and activity and clarity of 50% of the eyes had almost clear lenses; in contrast, 100% of the negative control eyes lead to dense nuclear opacity. From the existing study, it is evident that alpha lipoic acid protects the lens against oxidative stress. These results in glucose induced cataracts in vitro studies not only exhibit the protective effect of alpha lipoic acid but also indicate that it prevents cataractogenesis by virtue of its antioxidant properties. Alpha lipoic acid therefore, may be useful for prophylaxis or treatment against cataract. After 72 hr. incubated in glucose 55 mM, lens becomes entirely opaque as against lenses in normal control. Incubation of lenses with alpha lipoic acid and ascorbic acid both the concentrations were used, which seem to retard the progression of opacification compared with lenses incubated in glucose 55 mM (Negative Control). The effect of alpha lipoic acid, on the positive control groups showed considerable retardation in the progression of lens opacification which is near normal when compared to negative control.

CONCLUSION

The Present study suggests that Alpha lipoic acid treated groups have been revealed to increase the content of water-soluble proteins, retard the progression of cataractogenesis initiated by high glucose concentration. Alpha lipoic acid possesses anti-cataract activity due to the presence of antioxidant properties which might be helpful in preventing cataract formation.

The probable mechanism action of alpha lipoic acid involved in anti-cataract activity may be because of the following reasons,

- Alpha lipoic acid inhibits advanced glycation and glyco-oxidation due to reduction of oxidative stress of lens and also the end products of glycation like protein, lipid, nucleic acid produced during the development of cataract.
- Due to good anti-oxidant property alpha lipoic acid also prevents production of reactive oxygen species, which in turn inhibits cell proliferation, apoptosis, cell dysfunction and produces anti-cataract effect.

Alpha lipoic acid may also increase water content of lens protein that may contribute in the prevention of cataractogenesis.

Further investigations on the mechanism of action of Alpha lipoic acid are required and may have a considerable impact on future clinical treatments of patients with cataract diseases.

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