

Anticataract Activity of Triphala by using Invitro Goat Lens Model

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<https://doi.org/10.55041/ijst.v2i4.369>

Cite this Article: Gunjal, G. & V.P, M. (2026). Anticataract Activity of Triphala by using Invitro Goat Lens Model. International Journal of Science, Strategic Management and Technology, 02(04). <https://doi.org/10.55041/ijst.v2i4.369>

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ABSTRACT:

Cataracts are a leading cause of blindness and visual impairment worldwide, with oxidative stress and aging being major contributing factors. Triphala, a traditional Ayurvedic polyherbal formulation, has been reported to possess antioxidant, anti-inflammatory, and rejuvenating properties. This study aimed to evaluate the anticataract activity of Triphala on goat lenses. Goat lenses were treated with Triphala extracts at different concentrations (15µg/ml, 30µg/ml, and 60µg/ml) and compared with normal, negative control (glucose 55 mM), and positive control (ascorbic acid 40µg/ml) groups. Total proteins and water-soluble proteins were estimated in all groups. The results showed that Triphala extracts significantly prevented protein aggregation and maintained lens transparency, indicating its potential anticataract activity. These findings suggest that Triphala may be a useful adjunct in the prevention or treatment of cataracts. Further studies are warranted to explore the clinical applications of Triphala in ophthalmology.

KEYWORDS: Triphala, Catract, lense culture.

INTRODUCTION:

The dynamic component of the eye's optical system, the crystalline lens, is located behind the iris and is in charge of focusing the picture onto the retina. Any loss of transparency or lens opacities are characteristics of cataracts. The most typical signs of cataracts include glare, color disturbance, diminished contrast sensitivity, and vision impairment. In the general population, changes in the lens may also be indicators of aging and systemic health. Three conventional types of cataracts are distinguished based on the type of lens opacities: cortical, posterior subcapsular, and nuclear. In addition to being related to one another, these categories can lead to complete lens opacification if left untreated. The most frequent causes of cataracts in adults include trauma, age, diabetes, steroid usage, and family history. There are a lot of congenital cataracts.

The most common cause of blindness and visual impairment in the world is still cataracts. The risk of cataracts is known to be increased by a number of risk factors, including aging, diabetes, sunlight, environmental factors, smoking, dietary deficiencies or inadequacies, and a lack of antioxidant consumption.



Cataract can be caused by a number of factors, including aging, prolonged sun exposure, smoking, excessive cholesterol and triglycerides, diabetes, certain myopia-related eye disorders, and retinopathy. Cataract can also result from acute eye damage, renal illness, and hypertension.

Since ancient times, a wide range of illnesses have been treated with triphala, a well-known polyherbal medication. In his literature, Acharya Charaka stated that a person can live for a hundred years by consuming Triphala daily for a year. This is similar to Rasayana.

According to the Ayurvedic Formulary of India, triphala is a blend of three fruits made up of dried versions of *Terminalia belerica* Linn (Combretaceae), *Terminalia chebula* (Combretaceae), and *Emblica officinalis* Gaertn (Euphorbiaceae) in equal amounts (1:1:1).

Properties of triphala in Ayurveda Rasa: Rasa Kashaya Pradhana Rasa Pancha Guna: Guru and Ruksha (modest) Madhura Doshagnata: Virya: Ushna Vipaka: mostly employed in cases with mildly disordered Pitta, moderate vitiation of Vata, and predominance of Kapha dosha.

INDIVIDUAL COMPONENT OF TRIPHALA:

Emblica officinalis (Amalaki):

Individual chemical components: tannins, riboflavin, nicotinic acid, vitamin C, and carotene.

• *Terminalia chebula* (Hiritaki):

Individual chemical components: polyphenolic chemicals, anthraquinones, and tannins.

• *Terminalia belerica* (Bibhitaki):

Individual chemical components: glycosides, tannic acid, and gallic acid.

COMPOSITION OF TRIPHALA POWDER:

1. AMALAKI:

BOTANICAL GROUP:

- Kingdom : Plantae
- Division: Angiospermae
- Class: Dicotyledonae
- Order: Geraniales
- Family: Euphorbiaceae

- Genus: Emblica



Fig.No.1. Amalaki

MATERIAL AND METHODS:

PLANT MATERIAL COLLECTION:

The drug's collection The triphala compound's ingredients, which include the fruits of Amalaki (*Emblica officinalis*), Bibhitaki (*Terminalia bellerica*), and Haritaki (*Terminalia chebula*), were purchased from the on-campus pharmacy. Correlating their morphological and microscopical properties with pertinent literature allowed for the confirmation of their characteristics.

PLANT EXTRACT PREPARATION:

fruits of *T. bellerica*, *T. chebula*, and *E. officinalis* were mechanically crushed and shade-dried. Using a Soxhlet extractor, plant extracts were made at 65 °C using several solvents, including petroleum ether, distilled water, and methanol. After filtering, the extracts were concentrated in a rotary evaporator at 45 to 50 degrees Celsius. The remaining semisolid materials were then lyophilized and kept at -20 degrees Celsius until they were needed again



Fig.No.2.Extraction Of Triphala

PHYTOCHEMICAL ANALYSIS:

COLLECTION AND EXTRACTION OF SPECIMEN:

Triphala is the Ayurvedic medication chosen for this investigation. We bought triphala powder from a nearby pharmacy. Five different solvents, including water, acetone, chloroform, methanol, and ethanol, were then used to extract the dried powdered materials. Ten grams of triphala powder and one hundred milliliters of distilled water were combined, heated for two hours, and then filtered in order to do an aqueous extraction. In contrast, ten grams of powdered materials were combined with 100 milliliters of each solvent separately in a mechanical shaker and allowed to sit at room temperature for 48 hours in order to create the acetone, chloroform, methanol, and ethanol extracts. For later usage, the extracts were filtered, concentrated, dried, and refrigerated at 4 °C.

LENS CULTURE:

Goat eye lenses used for the study were taken from a nearby slaughterhouse, transferred right away, and kept in the lab between 0 and 4 °C. The specimen was isolated using the extra capsular extraction technique and kept for 72 hours at room temperature and pH 7.8 in artificial aqueous humour (NaCl–140 mM, KCl–5 mM, MgCl₂–2 mM, NaHCO₃–0.5 mM, NaH (PO₄)₂–0.5 mM, CaCl₂–0.4 mM, and Glucose–5.5 mM). To stop additional bacterial contamination, penicillin (32 mg) and streptomycin (250 mg) were added to the culture media. Only transparent lenses were employed for in-vitro studies; lenses that acquired damage with fake opacities were discarded

CARARACT FORMATION:

For cataract formation, a glucose solution with a 55 mM concentration was utilized. The sorbitol route metabolizes glucose at higher concentrations. Over-hydration and oxidative stress brought on by the buildup of polyol (sugar + alcohol) resulted in cataract formation. For 72 hours, each of these lenses was cultured in artificially generated aqueous humor containing varying concentrations of glucose.

STUDY DESIGN AND GROUPS:

Goat lenses were divided into six groups of six lenses each following Table 1.

Table No. 1: Treatment groups:

Group No.	Group Name	Treatment	Drug Dose
I.	Normal Control	Aq. Humor + Glucose 5.5 mM	-
II	Negative Control	Aq. Humor + Glucose 55 mM	-
III	Standard	Aq. Humor + Glucose 55 mM +Ascorbic Acid	40 µg/ml
IV	Test 1	Aq. Humor + Glucose 55 mM + Triphala	15 µg/ml
V	Test 2	Aq. Humor + Glucose 55 mM + Triphala	30 µg/ml

VI	Test 3	Aq. Humor + Glucose 55 mM + Triphala	60 µg/ml
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PREPARATION OF LENS HOMOGENATE:

Following a 72-hour incubation period, the lens was homogenized in tris buffer (0.23 M, pH 7.8) with 0.25×10^{-3} M EDTA. The homogenate was then adjusted to 10% w/v and centrifuged at 10,000 G for one hour at 4°C. The biochemical parameters were estimated using the supernatant. A homogenate in sodium phosphate buffer (pH-7.4) was made in order to estimate the amount of water-soluble proteins. **RESULTS AND DISCUSSION:**

IN- VITRO ANTICATARACT ACTIVITY:

After 8 hours of incubation with 55 mM glucose, opacification began to appear on the posterior surface of the lens at the periphery. Over the course of 72 hours, this gradually expanded toward the center and reached full opacification.

PHOTOGRAPHIC EVALUATION:

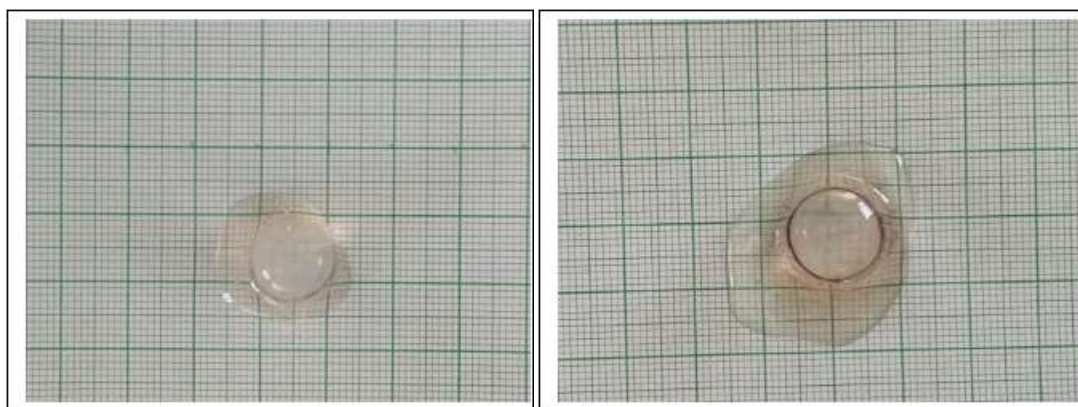


Fig no 3 (A): Normal control (Group I) Fig (B): Negative control (Group II)

in preventing the development of cataracts (fig. F) compared to Group IV (fig. D) and Group V [fig. (E)]. Group I (the normal control group) retained its transparency after 72 hours of incubation (fig. A), whereas Group II (the negative control group) completely lost it (fig. B), demonstrating full cataractogenesis. The lenses in Group III (positive control group) (fig.C) that received regular ascorbic acid treatment allowed the graph paper's squares to be seen through the lenses. Groups IV, V, and VI's goat lenses, which contained higher dosages of Triphala, were less cloudy, and the squares of the graph paper could be seen through the lenses, suggesting that cataract formation was suppressed [(D, E, and F)]. Group VI, which included 60 µg/ml, had greater efficacy.

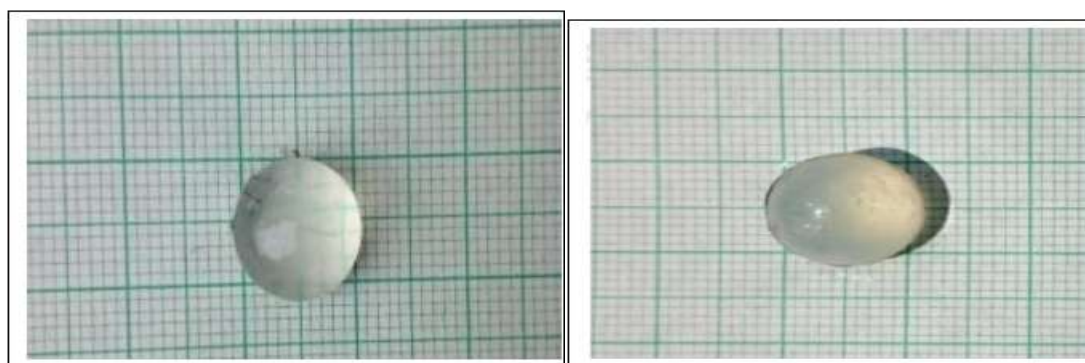


Fig (C) 4 : Positive control (Group III)

Fig (D): Group IV

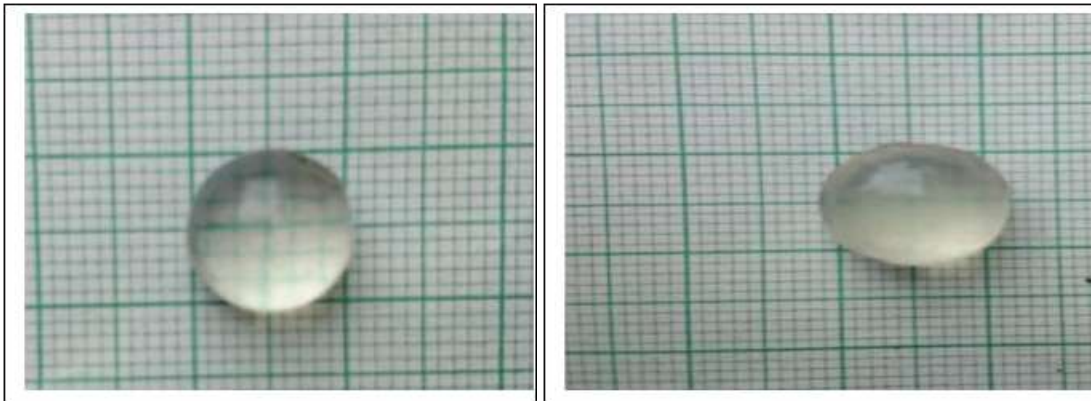


Fig (E) 5 : Group V

Fig (F): Group VI

Normal control (Group I):

As a normal control, the lens was incubated in aqueous humor with 5.5 mM glucose conc for 72 hours. Numerous squares are plainly visible through the transparent lens, which is unaffected by the low glucose concentration.

Negative control (Group II):

After the lens is incubated in aqueous humor for 72 hours with 55 mM of glucose, the lens produces a high concentration of glucose, which is metabolized via the sorbitol pathway and causes polyol to accumulate, leading to oxidative stress and hydration. Cataractogenesis results. There was thick, widespread opacity in the lens.

Standard(Group III):

The lens exhibited a modest degree of opacity after 72 hours of incubation in aqueous humor + 55 mM glucose + 40µg/ml ascorbic acid standard drug. Compared to test drug VI, the number of squares was not clearly apparent through the lens.

Test (Group IV and V):

The lens showed a modest degree of opacity after 72 hours of incubation in aqueous humor + 55 mM glucose + 15 µg/ml and 30 µg/ml Triphala test drug. Compared to Triphala 60 µg/ml test drug, the number of squares was not clearly visible through the lens.

Test (Group VI):

A number of squares were clearly visible through the lens after it had been incubated for 72 hours in aqueous humor plus 55 mM glucose and 60 µg/ml of the Triphala test medication. Because the test medication prevents cataract formation and oxidative stress, the lens displayed no opacity.

The in-vitro model for cataract induction with a glucose concentration of 55 mM works well on goat lenses that have been isolated. It has been demonstrated that incubating goat lenses in media with a high concentration of glucose (55 mM) causes cataract development and a significant decrease in Na⁺/K⁺-ATPase activity as opacity increases. The accumulation of Na⁺ and loss of K⁺ due to hydration and swelling of the lens fibers caused by the inhibition of Na⁺/K⁺-ATPase results in cataractogenesis. Lens opacification results from a decrease in total proteins due to this shift

in the Na⁺, K⁺ ratio, which also affects the protein content of the lens. Because triphala corrects abnormalities in the polyol pathway by lowering intracellular glucose, sorbitol concentration, and aldose reductase activity, the imbalance of Na⁺ and K⁺ was avoided.

Table No. 2: Effect of Triphala 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 1 (Triphala 15µg/ml)	2
5.	Test 2 (Triphala 30µg/ml)	1
6.	Test 3 (Triphala 60µg/ml)	0

Normal control: A clear lens is produced with zero degree opacity.

Negative control: a large amount of thick opacity due to a high prevalence of glucose-induced cataractogenesis.

Clear lenses were not discovered in the positive control (ascorbic acid 40µg/ml), which shows a modest degree of opacity.

In Test 1 (Triphala 15µg/ml), clear lenses were not discovered; instead, lenses displayed a modest degree of opacity.

Test 2 (Triphala 30µg/ml): no clear lens was detected, however the lenses exhibit a modest degree of opacity.

Test 3 (Triphala 60µg/ml): A clean lens is achieved with zero degree opacity. The test medication prevents cataract development.

Using a goat eye lens model, triphala was tested for in vitro anticataract activity. Here, triphala serves a variety of biological purposes, including anticataract action. 50% of the eyes had nearly clear lenses, whereas 100% of the negative control eyes acquired dense nuclear opacity. This indicates that triphala greatly protected the lens morphology, activity, and clarity. It is clear from the current study that triphala shields the lens from oxidative damage. These findings from in vitro research on glucose-induced cataracts show that triphala has a preventive impact and that its antioxidant qualities also stop cataractogenesis.

Therefore, triphala may be helpful for cataract prevention or treatment. In contrast to lenses in normal control, the lens becomes totally opaque after 72 hours of incubation in glucose 55 mM. In contrast to lenses incubated in glucose 55 mM (Negative Control), triphala and ascorbic acid concentrations were utilized to incubate the lenses, which appear to slow the progression of opacification. When compared to the negative control, the effect of triphala on the positive control groups revealed a significant delay in the development of lens opacification, which is close to normal.

Table.No.3: Effect of Triphala on protein levels (total proteins and water-soluble proteins) in goat lens homogenate after 72 hours of incubation in glucose 55 mm induced cataract:

Sr.No.	Treatment	Total proteins	Water soluble
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		[mg/gm]	proteins[mg/gm]
1.	Normal	246.82 ± 3.421	120.39 ± 2.831
2.	Negative control (Glucose 55 mM)	191.39 ± 4.192	90.12 ± 3.421
3.	Positive control (Ascorbic acid 40µg/ml)	261.91 ± 2.831	135.91 ± 2.392
4.	Test 1(Triphala 15µg/ml)	229.45 ± 3.912	105.18 ± 3.219
5.	Test 2 (Triphala 30µg/ml)	244.18 ± 3.219	119.45 ± 2.951
6..	Test 3 (Triphala 60µg/ml)	257.63 ± 2.951	129.82 ± 2.641

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 Dennett test) N.S. –nonsignificant.

In comparison to normal lenses (Group I), lenses treated with glucose 55 mM (Group II) exhibited considerably lower amounts of proteins (both total and water-soluble proteins) in the lens homogenate ($P < 0.01$). When compared to lenses treated with glucose 55 mM (Group II), lenses treated with ascorbic acid (Group III) and lenses treated with triphala (Group IV, V, and VI) exhibited greater protein (total and water-soluble protein) concentrations ($P < 0.01$).

CONCLUSION:

The present study was undertaken to evaluate the anticataract potential of Triphala using the goat eye lens model exposed to high glucose-induced cataractogenesis. Cataract formation is commonly associated with increased glucose levels, which lead to oxidative stress and protein insolubilization in the lens. In our study, the Triphala-treated group demonstrated a significant increase in the water-soluble protein content of the lens, indicating its protective effect against lens opacification and protein denaturation.

The observed anticataract activity of Triphala may be attributed to its rich antioxidant properties, which play a crucial role in neutralizing reactive oxygen species (ROS) and preventing oxidative damage to lens proteins. Furthermore, Triphala appears to inhibit the key factors responsible for cataract formation, thereby maintaining lens clarity and protein solubility. The phytochemical constituents of Triphala, such as tannins, flavonoids, and phenolic compounds, are likely contributors to this protective effect due to their well-known antioxidative and anti-glycation properties.

Overall, the findings of this research suggest that Triphala has promising anticataract activity and could be considered as a potential natural therapeutic agent for the prevention or delay of cataract development, especially in diabetic conditions. However, further studies including in vivo models and clinical trials are essential to confirm its efficacy and safety in human subjects.



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