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PHYTOCHEMICAL SCREENING AND *IN-VITRO* ANTI-CATARACT ACTIVITY ON THE SEED OF *CUCUMIS MELO* L. ETHANOLIC EXTRACT

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Keywords:

Anti-cataract, Goat lens, Antioxidant, Vitamin A, Artificial Aqueous Humor, Water Soluble Proteins

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ABSTRACT: Objective: Evaluation of *in-vitro* anti-cataract activity on the Cucumis melo L ethanolic extract seeds by glucose-induced cataract in incubated goat lenses. **Methods:** Anticataract activity is performed by using an isolated goat lens. The goat lens was divided into six groups. Group I: Lens was incubated in artificial aqueous Humor (Glucose 5.5 mM). Group II: Lens was incubated with artificial aqueous humor (Glucose 55 mM). Group III: Lens was incubated in artificial aqueous Humor (Glucose 55 mM) + 40 µg/ml of Ascorbic Acid; Group IV, V, and IV: Lens was incubated in artificial aqueous humor (Glucose 55 Mm) + 20 µg/ml, 40 µg/ml and 60 µg/ml of ethanolic extract of seeds on *Cucumis melo* L respectively and subjected to photographic evaluation for opacity; The lens was homogenized using tris buffer, and total protein and water-soluble proteins content were determined. **Results:** Photographic examination of the eyes showed that treatment with ethanolic extracts of the seeds of *Cucumis melo* L retarded the progression of lens opacification. Vitamin A, Ascorbic acid, β-carotene, Carotenoids, and Flavonoids these phytoconstituents possess antioxidant properties to reduce the development of cataracts. The total and Water soluble protein's activity is increased in the extract-treated lenses compared with the standard drug ascorbic acid. **Conclusion:** From this study, we conclude that ethanolic extract of the seeds of *Cucumis melo* L significantly reduced cataracts at the dose of 80µg/ml in goat lens.

INTRODUCTION: Clouding of the eye's lens is called a cataract. This haziness can lead to vision loss and, eventually, blindness. One or both eyes may be harmed by cataracts, which frequently advance slowly. A cataract forms when proteins in the eye group together and prevent the lens from sending clear images to the retina. The retina works by analyzing electrical impulses from light that passes via the lens.

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It sends impulses, which the optic nerve picks up and sends to the brain. It worsens over time until it obstructs your vision. You can get cataracts in both of your eyes, but they usually don't happen at the same time. Cataracts commonly occur in elderly people [1].

A cataract is associated with old age. A cataract is a major complication of diabetes Mellitus because higher glycosylated hemoglobin levels are significantly associated with an increased risk of cataracts. However, several factors have been shown to cause cataracts. There is yet no knowledge of the metabolic basis of cataractogenesis. It is a multi-factorial disease that occurs mainly due to the formation of large protein

aggregates in the lens. The lens Na - K+- ATPase activity plays an important role in maintaining lens transparency and impairment causes the accumulation of Na+ and loss of K+ with hydration and lens fiber swelling that causes cataractogenesis [2].

The normal lens contains containing glutathione and ascorbic acid as antioxidants. With the increase in age, this anti-oxidative mechanism becomes less effective. An increase in inactive insoluble proteins and semi-permeability of the lens capsule may lead to cataract formation. The pathophysiology of cataracts is not fully understood. Three metabolic pathways convert glucose into energy (ATP) and other relevant metabolic molecules. They are glycolysis, pentose phosphate shunt, and polyol pathway [3].

The development of plant-based anti-cataract drugs has been given importance in the global market. Many plants have not also been far subjected to scientific evaluation. *Cucumis melo* L is a dicotyledonous plant belonging to the Cucurbitaceae family. The plant is locally known as "Mulam palam or Muskmelon".

The nutritionally important compounds like phenolic, flavonoids, alkaloids, sterols, terpenoids, glycosides, and many other metabolites have been isolated from different parts of *Cucumis melo* Land possess various pharmacological activities, including antioxidant, antimicrobial agents, anti-inflammatory, anti-ulcer activity, Antidiabetic activity, Diuretic activity, Anti-fertility activity, Anthelmintic activity, Analgesic activity, Hepatoprotective activity [4, 5].

Hence, this study has evaluated anti-cataract activity on the seed of *Cucumis melo* L ethanolic extract on glucose-induced cataract in goat lens, and ascorbic acid served as standard. Glucose-induced cataract in the goat lens model was practiced to assess the inhibition of cataract formation.

MATERIALS AND METHODS:

Plant Collection and Authentification: The seeds of *Cucumis melo* L were collected from Sri Kumaran Agro Centre, Tharamangalam, Salem. The plant material was authenticated by Dr. D. Stephen, M.Sc., Ph.D., Assistant Professor, Department of Botany, The American College, Madurai - 625002.

Preparation of Plant Extracts [6, 7]: Maceration generally consists of several steps for small-scale extraction. Firstly, grinding plant materials into small particles was used to increase the surface area for proper mixing with solvent. In this process, 500 gm of the seed of *Cucumis melo* L was coarsely powdered, then it was placed in an individual closed vessel with the ethanol and allowed to stand at room temperature for at least three days with frequent agitation until the soluble matter was dissolved. Then the mixture is strained, damp solid material is pressed and the combined liquids are clarified by filtration or decantation after standing.

Phytochemical Analysis [8]: The Ethanolic seed extract of *Cucumis melo* L was subjected to preliminary phytochemical screening to reveal the presence of various phytoconstituents according to the methods outlined in Khandelwal 2008.

***In-vitro* Anti-Cataract Activity by Glucose-Induced Cataract in Incubated Goat Lenses Model [9-13]:**

Materials Requirements: Goat lenses, Sodium chloride, Potassium chloride, Magnesium chloride, Sodium bicarbonate, Sodium phosphate, Calcium chloride, Glucose, Penicillin G, Streptomycin Ascorbic Acid and Ethanolic extract of seed of *Cucumis melo* L.

Collection of Eye Balls: In the current investigation, goat eyes were used. They were obtained from the slaughterhouse. Immediately after slaughter and transported to the laboratory at 0-4^{o}C.

Preparation of Lens Culture: The slaughterhouse brought fresh goat eyeballs that were then transported to the laboratory at 0-4 °C. Extracapsular extraction was used to remove the lens, which was then cultured in artificial aqueous humor (NaCl140 mM, $MgCl_2$ 2 mM, $NaHCO_3$0.5 mM, $NaHPO_4$ 0.5 mM, $CaCl_2$ 0.4 mM, KCl 5 mM, and glucose 5.5 mM) 5 mM) at ambient temperature and add NaHCO3 to maintain pH 7.8. Penicillin G 32% and streptomycin 250 mg% were added to the culture media to prevent bacterial contamination. Glucose in the lens was metabolized at high concentrations through sorbitol

pathway and polyol accumulation, causing overhydration and oxidative stress. As a result, cataractogenesis begins.

Induction of Cataracts on Goat Lenses: Glucose at a concentration of 55 mM was used to induce cataracts. The lens's high glucose concentrations can use the sorbitol pathway to break it down. Accumulation of polyol (sugar alcohols) causes over-hydration and oxidative stress. This leads to cataractogenesis. These lenses were incubated in artificial aqueous humor with different concentrations of glucose (5.5 mM) served as normal control, and 55 mM served as toxic control) for 72 hours.

Experimental Design: Goat lenses were divided into six groups containing one lens in each and incubated as following **Table 1.**

TABLE 1: EXPERIMENTAL DESIGN OF *IN-VITRO* ANTI-CATARACT ACTIVITY

Group	Treatment
I	Goat lens + Artificial Aq. Humor (Glucose 5.5 mM)
II	Goat lens + Artificial Aq. Humor (Glucose 55 mM)
III	Goat lens + Artificial Aq. Humor (Glucose 55 mM) + 40 µg/ml of Ascorbic Acid
IV	Goat lens + Artificial Aq. Humor(Glucose 55 Mm) + 20 µg/ml of Ethanolic extract of seeds on *Cucumis melo* L
V	Goat lens + Artificial Aq. Humor (Glucose 55 mM) + 40 µg/ml of Ethanolic extract of seeds on *Cucumis melo* L
VI	Goat lens + Artificial Aq. Humor (Glucose 55 mM) + 80 µg/ml of Ethanolic extract of seeds on *Cucumis melo* L

Assessment of Anti-Cataract Activity:

Photographic Evaluation: To test lens opacity, lenses were put on a wire mesh with their posterior surfaces touching the mesh. The number of mesh squares visible through the lens was observed.

The opacity was graded using the following system.

0 - Means there is no opacity.
1 - A very slight amount of opacity.
2 - Diffuse opacity is present.
3. The presence of thick, widespread opacity

Analysis of Biochemical Parameter in Homogenate Lens:

Preparation of Lens Homogenate: After 72 hours of incubation, homogenate of lens 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 1 hour and the supernatant was used for the estimation of biochemical parameters.

Estimation of Total Protein Content: 4 ml of alkaline copper solution was added to 0. 1 ml of lens homogenate and allowed to stand for 10min. Then, 0.4 ml of phenol reagent was added rapidly, mixed quickly, and incubated at room temperature for 30 min for colour development. Readings were obtained at 610 nm in the UV spectrum against a distilled water-prepared blank. The protein content was calculated from a standard curve prepared with bovine serum albumin and expressed as µg/mg lens tissue.

Statistical Analysis: Results were expressed as Mean ± Standard Error of Mean (SEM).

RESULTS:

Phytochemical Analysis: Preliminary phytochemical analysis of Ethanolic extract of seeds on *Cucumis melo* Lrevealed the presence of various components like Alkaloids, Anthroquinone, Flavonoids, Steroids & Triterpenoids, Proteins, Carbohydrates and Phenol **Table 2.** The seeds of *Cucumis melo* Lis are also an important source of vitamin A, thiamine, riboflavin, niacin, ascorbic acid, β -carotene and many other functional compounds. The antioxidant activity of the Ethanolic extract of seeds on *Cucumis melo* L has been shown to offer protection against cataracts.

TABLE 2: PHYTOCHEMICAL ANALYSIS OF ETHANOLIC EXTRACTS SEEDS ON *CUCUMIS MELO* L

S. no.	Chemical Constituents	Ethanolic Extract
1	Alkaloids	+
2	Anthraquinone	+
3	Flavonoids	+
4	Saponins	-
5	Steroids & Triterpenoids	+
6	Proteins	+
7	Carbohydrates	+
8	Tannins	-
9	Phenol	+

Note: (+) Present (-) Absent.

In-vitro Anti-Cataract Activity by Glucose-Induced Cataract in Incubated Goat Lenses Model: Photographic Evaluation:

TABLE 3: EFFECT OF ETHANOLIC EXTRACT OF ETHANOLIC EXTRACT OF SEEDS ON *CUCUMIS MELO* LINDEGREE OF OPACITY ON GOAT LENS BY GLUCOSE-INDUCED CATARACT

S. no.	Group	Degree of opacity
1	I (Normal control)	0
2	II (Negative Control)	3
3	III (positive control) (Standard drug Ascorbic Acid - 40 µg/ml)	1
4	IV (Test 1 -20 µg/ml of Ethanolic extract of seeds on*Cucumis melo* L	2
5	V (Test 2 - 40 µg/ml of Ethanolic extract of seeds on *Cucumis melo* L	1
6	VI (Test 3 - 80 µg/ml of Ethanolic extract of seeds on *Cucumis melo* L	0

Normal Control: Zero-degree opacity occurred, clear lens was obtained.

Negative Control: The presence of extensive thick opacity because of high concentration of glucose-induced cataractogenesis.

Positive Control (Ascorbic acid 40µg/ml): The lens showed a slight degree of opacity, and a slightly clear lens was obtained.

Test 1 (20 µg/ml of Ethanolic Extract of Seeds on *Cucumis melo* L: The lens shows diffuse opacity, the clear lens was not found.

Test 2 (40 µg/ml of Ethanolic Extract of Seeds on *Cucumis melo* L: The lens shows a slight degree of opacity, a clear lens was not found.

Test 3 (80 µg/ml of Ethanolic Extract of Seeds on *Cucumis melo* L: The clear lens was obtained with zero degree opacity.

FIG. (A): NORMAL CONTROL (GROUP I)

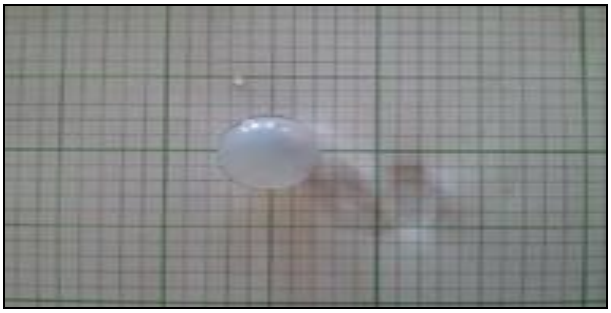

FIG. (B): NEGATIVE CONTROL (GROUP II)

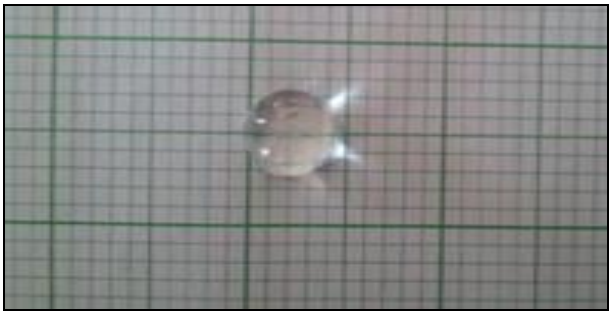

FIG. (C): POSITIVE CONTROL (GROUP III)

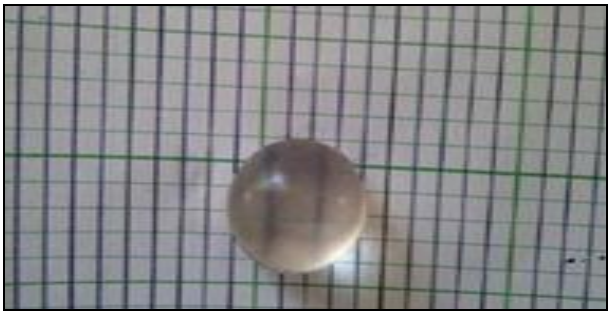

FIG. (D): TEST 1 -20 MG/ML(GROUP IV)

FIG. (E): TEST 2 - 40 MG/ML (GROUP V)

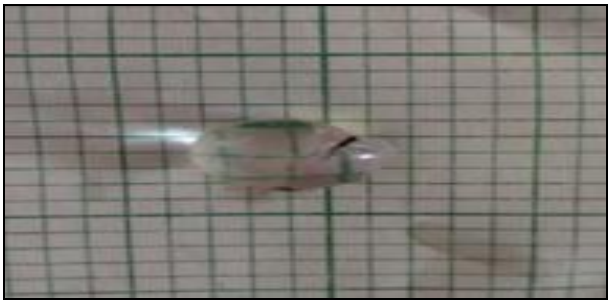

FIG. (F): TEST 3 - 80 MG/ML (GROUP VI)

FIG. 1: PHOTOGRAPHIC EVALUATION OF LENS OPACITY

Analysis of Biochemical Parameter in Homogenate Lens:

Estimation of Total Protein Content in Homogenate Lens:

TABLE 4: ESTIMATION OF TOTAL PROTEIN CONTENT IN HOMOGENATE LENS

S. no.	Group	Total Protein Content (mg/ml)
1	I (Normal control)	215.3±0.8
2	II (Negative Control)	163±0.5
3	III (positive control) (Standard drug Ascorbic Acid - 40 µg/ml)	192.6±0.6
4	IV (Test 1 -20 µg/ml of Ethanolic extract of seeds on *Cucumis melo* L.	171.3±0.8
5	V (Test 2 - 40 µg/ml of Ethanolic extract of seeds on *Cucumis melo* L.	184.3±0.8
6	VI (Test 3 - 80 µg/ml of Ethanolic extract of seeds on *Cucumis melo* L.	194±0.5

Values are expressed as Mean ± SEM. n=6

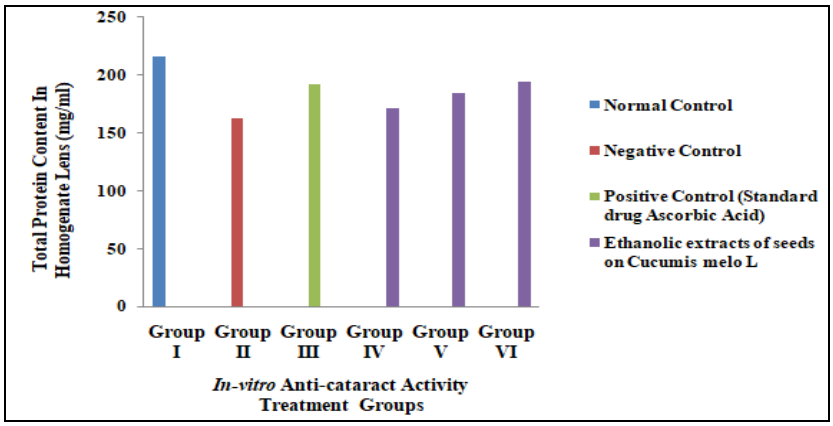

FIG. 2: ESTIMATION OF TOTAL PROTEIN CONTENT IN HOMOGENATE LENS

Estimation of Water-Soluble Protein Content in Homogenate Lens:

TABLE 5: ESTIMATION OF WATER-SOLUBLE PROTEIN CONTENT IN HOMOGENATE LENS

S. no.	Group	Water Soluble Protein Content (mg/ml)
1	I (Normal control)	82±0.5
2	II (Negative Control)	63.6±0.8
3	III (positive control) (Standard drug Ascorbic Acid - 40 µg/ml)	78.3±0.3
4	IV (Test 1 -20 µg/ml of Ethanolic extract of seeds on *Cucumis melo* L.	71.3±0.6
5	V (Test 2 - 40 µg/ml of Ethanolic extract of seeds on *Cucumis melo* L.	76.3±0.3
6	VI (Test 3 - 80 µg/ml of Ethanolic extract of seeds on *Cucumis melo* L.	81±0.5

Values are expressed as Mean ± SEM. n=6.

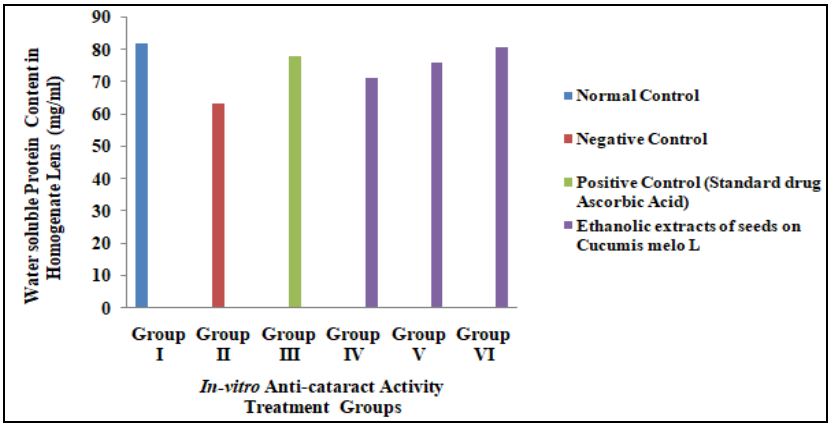

FIG. 3: ESTIMATION OF WATER-SOLUBLE PROTEIN CONTENT IN HOMOGENATE LENS

DISCUSSION: To access the inhibition of cataract by using Photographic evaluation to find out the degree of opacity on goat lenses. The results obtained are illustrated in **Table 3 and Fig. 1**.

After 8 hours of incubation with glucose 55 mM, lenses became transparent. This progressively increased towards the center, with complete opacification at the end of 72 hrs. In photographic evaluation, **Fig. 1**, After 72 hours of incubation, transparency was maintained in Group I (normal control group) **Fig. A. Still,** there was a complete loss of transparency in Group II (negative control group) **Fig. B,** indicating complete cataractogenesis. Group III (positive control group) **Fig. C** containing lens treated with standard ascorbic acid where squares of the graph paper were visible through the lenses. Group IV, V, and VI containing lens treated with Ethanolic extract of seeds on *Cucumis melo* L were respectively 20µg/ml, 40µg/ml,80 µg/ml and squares of the graph paper were visible through the lenses indicating suppression of cataract formation **Fig. D, E** and **F**. Group VI was more effective in suppressing cataract formation than Group IV and Group V **Table 3**.

The lens showed the absence of opacity because the Ethanolic extract of seeds on *Cucumis melo* L inhibits cataractogenesis and oxidative stress. Incubation of goat lenses in the media containing high glucose (55 mM) concentration has induced cataract. It has been shown to cause a considerable drop in Na^+/ K^+- ATPase activity, with the progression of opacity. The impairment of Na^+/ K^+-ATPase causes accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibers leading to cataractogenesis. This alteration in the Na^+, K^+ ratio changes the protein content of the lens, leading to a decrease in total proteins causing lens opacification. The imbalance of Na^+ and K^+ was prevented due to an action of Ethanolic extract of seeds on *Cucumis melo* L, which corrects imbalances in the polyol pathway by decreasing aldose reductase activity, sorbitol concentration, and intracellular glucose.

From **Table 4** and **Fig. 2**, the Ethanolic extracts of seeds on *Cucumis melo* L. significantly increase the concentration of total protein content in homogenate lens when compared to the negative control group and ascorbic acid standard drug. The standard drug showed a maximum increase in the concentration of total protein content in homogenate lens of 192.6±0.6 at 40mg. However, the Ethanolic extracts of seeds on *Cucumis melo* L. showed a significant increase in the concentration of total protein content in the homogenate lens of 194±0.5 at 80 mg. It was found to be higher than that of the standard.

From **Table 5** and **Fig. 3**, the Ethanolic extracts of seeds on *Cucumis melo* L. significantly increase the concentration of water-soluble protein content in the homogenate lens compared to the negative control group and ascorbic acid standard drug. The standard drug showed a maximum increase in water-soluble protein concentration in the homogenate lens of 78.3±0.3 mg/ml at 40mg.

Though the Ethanolic extracts of seeds on *Cucumis melo* L. showed a significant increase in the concentration of water soluble protein content in a homogenate lens of 81±0.5 mg/ml at 80 mg. It was found to be higher than that of the standard.

CONCULSION: Compared with the standard drug Ascorbic acid, ethanolic extract of seeds on Cucumis melo Lpossess anti cataract activity. This may be beneficial in the treatment of cataracts. Further studies can be carried out to *in-vivo* anti-cataract activity and elucidate the mechanism of action of Ethanolic extract of seeds on *Cucumis melo* L. This may be followed and clinical studies to establish its efficacy in humans.

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