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Research Article

EVALUATION OF ANTICATARACT ACTIVITY OF *SYZYGIUM CUMINI* SEEDS

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A B S T R A C T

The present investigation was aimed to evaluate the efficacy of different extracts of *Syzygium cumini* seed powder against glucose induced cataract in goat and chick lens. In this in vitro study goat lenses and chick lenses were subjected to photographic evaluation and goat lenses were subjected to biochemical parameters such as total proteins, water soluble proteins, Malondialdehyde, glutathione and total ATPases and catalase levels. Photographic examination of the eyes showed that treatment with different extracts of *Syzygium cumini* seed powder retarded the progression of lens opacification. The total proteins, water soluble proteins and total ATPases activity is increased in the extract treated lenses when compared with the standard drug. The catalase activity of the extract treated groups is increased when compared with the standard drug. The MDA levels were significantly lower in the lenses treated with the *Syzygium cumini* seed extracts. The glutathione levels in high glucose (55mM), compared to the normal control group was significantly low but *Syzygium cumini* treated groups showed higher levels of glutathione. These results support the view that ethanolic, petroleum ether and aqueous extract of seed *Syzygium cumini* as seen in the in vitro model may counteracts the effects of glucose in inducing cataract to some extent.

INTRODUCTION

Medicinal plants are part and parcel of human society to combat diseases, from the dawn of civilization[1]. Medicinal plants can bean important source of previously unknown chemical substances with potential therapeutic effects.

Scientific studies on a good number of medicinal plants indicate that promising herbal drugs can be developed for many health problems.

Cataract Definition

Cataract, the clouding of the lens develops later in the life and it is most likely the consequence of decades of accumulated damage to long lived lens protein. It is the most common cause of blindness. Visual loss occurs because of opacification of the lens obstructs light from passing and being focused onto the retina[2-4].

The cataract formation involves the wide range of mechanisms for some instances the oxidative mechanism also plays an important role in biological phenomena, including cataract formation. The formation of superoxide radicals in the aqueous humor in lens and its derivatization to other potent oxidants may be responsible for initiating various toxic biochemical reactions leading to opacification. The aldose reductase enzyme also plays an important role in pathogenesis of cataract.

Pathophysiology

Changes in the lens proteins (Crystalline) affect how the lens refracts light and reduce its clarity, therefore decreasing visual activity. Chemical modifications of these lens proteins lead to the change in lens colour. New cortical fibres are produced concentrically and lead to thickening and hardening of the lens in nuclear sclerosis, which often appears yellow and can increase the focusing power of the natural lens. Increasing myopia can also be evidence of a progressing nuclear sclerotic cataract.

Cortical cataracts are most often seen as whitish spokes peripherally in the lens, separated by fluid. Vacuoles and water clefts can also be seen in these lenses. Posterior sub capsular cataracts are due to the migration and enlargement of lens epithelial cells. Diabetes mellitus is a major factor in the formation of this type of cataract. Oxidative stress was connected with slow developing cataracts.

General Mechanism of cataract formation

The extreme opacification affects the transparent nature of lens. Lens transparency is necessary to enter incoming light passes through lens and falls on retina to form image.

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Opacification of lens causes light scattering, as light passes through lens and then to the retina where diminished focus of light impairs vision. The visual activity is lost in cataract due to the absorption of light by less transparent lens. The generalized mechanism of cataract includes lens fibre cells disruption, cellular protein aggregation and lens cell cytoplasm dysfunctioning.

Plant Introduction

Numerous medicinal plants are present in a collection of herbal preparations of the Indian traditional health care system (Ayurveda) named Rasayana. From the ancient time, plants have been playing a key role for the betterment of mankind presenting as an extraordinary source of natural medicine. The complexity in formulating chemical based drugs as well as their health related side effects and uprising cost has led worldwide researchers to focus on medicinal plant research. Bangladesh has a vast repository of diverse plant species where about five thousand plants species have been claimed as having significant medicinal values.

Syzygium cumini belongs to the family Myrtaceae. Commonly it is known as an amblang, Jambul, Jambolan and Kala Jamun in India. *Syzygium cumini* is recommended as a safe drug in various diseases by health organisations in the world[5].

Syzygium Cumini Seeds

The seed is used as an alternative natural healing system in the Ayurvedic, Unani and Chinese medicines. Bark of *S. cumini* yields a brown dye due to its high tannin content which is used in tanning leather and preserving fishing nets. The seeds and bark are well known in the Far East for the treatment of dysentery and in control of hyperglycaemia and glycosuria in diabetic patients. The astringent bark may be used as a gargle. Fruits are used as a relief for colic, while the wood yields a sulphate pulp that has medicinal uses.

Scientific Classification[6]

- Kingdom: Plantae
- Unranked: Angisperms
- Unranked: Eudicots
- Unranked: Rosids
- Order: Myrtales
- Family: Myrtaceae
- Genus: Syzygium
- Species: Cumini
- Binomial name: Syzygium cumini (L) Skeels.

Parts Used*:* seeds, leaves, fruits, bark[7].

Ayurvedic properties[8-13]*:*

- Rasa - Kasaya, Madhura ,Amla.
- Virya - Sita
- Guna- Laghu, Ruksa.
- Vipala- Madhura, Katu.
- Karma– Vatala, Pittahara, Kaphahara, Vistambhi, Grahi.

Phytochemistry

'Phyto' is the Greek word for plant. There are many families of phytochemicals and they help the human body in a variety of ways. Phytochemicals may protect human from a host of diseases. Phytochemicals are non-nutritive plant chemicals that have protective effects and disease preventive properties.

Fruit of *Syzygium cumini* contains Malic acid is the major acid (0.59gm of the wt of fruit), a small quantity of oxalic acid is also reported to be present. Gallic acid and tannins account for astringency of the fruit. The Fruit of *Syzygium cumini* is purple in colour due to the presence of cyaniding diglycosides[14].

Syzygium cumini seed powder

Brown coloured shows a few parenchymatous cells and numerous oval, rounded starch grains, measuring 7-28 μ in diameter.

Syzygium cumini seeds are extensively used for various ailments such as anti-inflammatory, hypolipidaemic, antidiabetic and antioxidant, neuroprotective and recently it has been reported for the DNA protection against radiation[6].

Preparation of Extracts

The *Syzygium cumini* Skeels fruits were first washed well and pulp was removed from the seeds. Seeds were washed several times with distilled water to remove the traces of pulp from the seeds. The seeds and leaves were dried at room temperature and coarsely powdered. The powders were taken equally and extracted with hexane to remove lipids. It was then filtered and the filtrate was discarded. The residue was subjected to successive solvent extraction with Petroleum ether, Ethyl acetate, Ehanol, Methanol and Water using Soxhlet extraction method, aqueous extract was prepared by cold maceration process. The Plant Extracts Were Concentrated Using Rotary Flash Evaporator (Buchi, Switzerland) And Stored In Desiccators.

Phytochemical Screenings

The seed extracts of *Syzygium cumini*were analysed for the presence of alkaloids, glycosides, tri terpenoids, steroids, saponins, flavonoids, tannins and carbohydrates according to standard methods[15].

Test for Alkaloids

2ml of dilute hydrochloric acid was added to the 5 ml of extract then treated with Dragondroff's reagent, appearance of an orange brown precipitate showed the presence of alkaloids.

Test for Glycosides

The extract was hydrolysed with dilute hydrochloric acid for few hours on a water bath. 1ml of pyridine and a few drops of sodium nitroprusside solution were added. Then 2-3 drops of dilute NaOH was mixed. Pink colour produced which turn into red indicated presence of glycosides.

Test for Triterpenoids

About 5 ml of extract was mixed in 2 ml of chloroform; 2 ml of acetic anhydride and a few drops of conc. H2SO4 were added. Reddish violet colours indicated the presence of triterpenoids.

Test for Steroids

10ml of chloroform was mixed with 2ml of extracts and conc. H2SO4 was added to form lower layer. A reddish yellow colour at the interface was an indicative of the presence of steroidal ring.

Test for Saponins

15 ml of distilled water was added to the extract and shaken vigorously until formation of a stable persistent froth which indicates presence of saponins

Test for Flavonoids

Few drops of dilute NaOH was mixed with 2 ml of extract. A yellow solution that turns colourless showed the presence of flavonoids.

Test for Tannins

In a test tube containing little quantity of extract few drops of 1 % lead acetate were added.Yellow precipitate appeared it showed the presence of tannins.

Test for Carbohydrates

The small portion of extract was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. After that 2ml of concentrated H2SO4 was poured carefully along the side of the test tube. Violet ring at the interphase was not formed which indicates absence of carbohydrate.

These are the phytochemical tests used for the evaluation of phytochemical parameters in *syzygium cumini*.

The effect of *Syzygium cumini* and its phytochemicals should also be investigated for its anti-diabetic activity and chemo-preventive effects in other models of carcinogens, that includes chemical, radiation and viral carcinogenesis models. Mechanistic studies responsible for the chemo-preventive and radio-protective effects are also lacking and need to be studied in detail.

Based on these facts these review high-lights the role of *Syzygium cumini* seeds in various treatments and recommend that further phytochemical and clinical research should be done on this traditional medicinal plant for the discovery of safer drugs. Studies should also be on understanding which of the phytochemicals are responsible for the observed beneficially effects.

Although most of the studies of *Syzygium cumini* as anti-diabetic agent with its possible mechanism of action and delaying complications of diabetes such as cataract, neuropathy have been conducted but detailed research on isolation of bio-actives through clinical trials followed by standardisation is seriously required to know potential of plant. Most of the pharmacological work was carried out on seeds of *Syzygium cumini* but the pharmacological potential of other parts also required to be explore.

MATERIALS AND METHODS

Sample Collection

The seeds of *Syzygium cumini* were collected from the premises of S.V University. The *Syzygium cumini* skeels fruits were first washed well and pulp was removed from the seeds. Seeds were washed several times with distilled water to remove the traces of pulp from the seeds. The collected seeds were air dried at room temperature in the department until constant weight was attained.

The dried seeds were pulverized into coarse powder using electric blender.

Solvent extraction

A portion of dried seed powder was soaked in the conical flask containing petroleum ether and wrapped with aluminium foil for 72 hours with occasional shaking. After 72 hours the extract was filtered using Whatman filter paper No:1. After the completion of the petroleum extraction process the concentrated powder is again extracted with methanol and water by cold maceration process same as like petroleum extraction process.

Eye balls

Goat eye balls were used in the present study. They were obtained from the slaughter house immediately after slaughter and transported to laboratory at 0-4 degree Celsius.

Preparation of lens culture

The lenses were removed by extra capsular extraction and incubated in artificial aqueous humor.

The artificial humor is prepared as follows

- NaCl: 140mM
- Kcl: 5mM
- $Mgcl_2$: 2mM
- $NaHCO_3$: 0.5mM
- $NaH(PO_4)_2$: 0.5mM
- $CaCl_2$: 0.4mM
- Glucose: 5.5mM.

Aqueous humor is prepared at room temperature and pH is 7.8 for 72 hours. The Pencillin- 32mg% and Streptomycin-250mg% were added to the culture media to prevent bacterial contamination.

Glucose in a concentration of 55mM was used to induce cataract.

Induction of invitro cataract in chick lens

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

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Preparation of lens homogenate

After incubation, lenses were homogenized in 10 volumes of 0.1 M Potassium phosphate buffer. The pH is adjusted to 7.0, then the homogenate was centrifuged at 1000rpm for 1hour and the supernatant was used for estimation of biochemical parameters.

Experimental design

The Anti-cataract activity was carried out with the extracts of *Syzygium cumini* seeds. The extract was taken in different doses.

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

The grouping of goat and chick lens with petroleum ether, aqueous and ethanolic extract is as follows

Group I: Glucose5.5mM (normal control)
Group II: Glucose55mM (toxic control)
Group III: Glucose 55mM+ Standard drug+ Lens culture
Group IV: Glucose55mM+ Extract 50µg/ml+ Lens culture
Group V: Glucose55mM+ Extract 100µg/ml+ Lens culture
Group VI: Glucose55mM+ Extract 150µg/ml+ Lens culture

Procedures

Morphological and Photographic Evaluation

Procedure

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

The degree of opacity was graded as follows:

- Absence of opacity: 0
- Slight degree of opacity: +
- Presence of diffuse opacity: ++
- Presence of extensive thick opacity: +++

Estimation of Total Protein Content

Total protein content was estimated by the Lowry method. The method is having the following procedure. 1% of the lens tissue was homogenized in 0.25M ice cold sucrose solution. 0.5 ml of the crude homogenate is thoroughly mixed with 1ml of Trichlor acetic acid. Then centrifuged at 1000 rpm for about 15 minutes. Discard the supernatant liquid. The pellet was dissolved in 1ml of 1N NaOH. Then 4ml of alkaline copper reagent was added followed by 0.4 ml of Folin- phenol Reagent (Folin reagent):(Distilled water){1:1}. Read the colorimeter at 600nm.

Chemicals Required

- 10% TCA: 10 grams of Trichloro acetic acid is dissolved in 100ml of distilled water.
- 1N NaOH: 4 grams of NaOH is dissolved in 100ml of distilled water.
- Alkaline copper reagent (ACR):
- SOLUTION A: 0.4 grams of NaoH was dissolved in 100ml of distilled water. Then 2 grams of Na_2Co_3 was added.
- SOLUTION B: 0.5 grams of $CuSo_4$ was dissolved in 100 ml of distilled water. Then 1 gram of sodium potassium tartarate was added.
- Solution A: 50 ml
- Solution B: 1ml
- Both the solutions were mixed properly.
- Folin Phenol reagent: These is prepared by making 1:1 dilution i.e. 1ml of folin phenol reagent: 1ml of distilled water.

Estimation of Catalase Activity

The following procedure is followed for the estimation of catalase activity.

Catalase activity was measured by a slightly modified version of Acbi (1984). 5% of the tissue homogenate was prepared in 50mM phosphate buffer (p^H 7.0) containing 0.1mM EDTA. Centrifuge at 10,000 RPM/ 10minutes at 0^0 C. Supernatant is used as an enzyme source. 10µl of 100% ethanol was added to 100µl of the tissue homogenate and then placed in an ice bath for 30 minutes (Tissue extract). The above solution is kept at room temperature then add 100µl of Triton-X100 RS.

Fill cuvette in spectrophotometer with

- 200µl of PO_4 buffer
- 50µl of tissue extract
- 250µl of 0.066M H_20_2 in PO_4 buffer

Decreases the O.D measured at 240nm for 60 seconds in U.V Spectrophotometer.

Chemicals

- 50mM Phosphate Buffer (p^H 7.0): 2.12 grams of dibasic potassium phosphate and 1.06 grams of monobasic salt are dissolved in 400ml distilled water.
- 0.1mM EDTA: 3.12 mg (0.00372) of EDTA dissolved in 100ml of distilled water
- 100% Ethanol
- 0.066M H_20_2: 0.740ml of H_20_2 is dissolved in 99.26ml of phosphate buffer.

Estimation of Glutathione Peroxidase

Procedure

Glutathione estimation can be done by the following procedure

- Tris buffer: 0.2ml
- EDTA: 0.2ml
- Sodium azide: 0.1ml
- Tissue homogenate: 0.5ml
- Glutathione: 0.2ml
- H_2O_2: 0.1ml

The above chemicals were added in the definite volumes that are provided in the above and mixed well. The mixture is incubated at 37^oC for minutes. The remixture is arrested by 0.5ml of 10% TCA and centrifuged at 3000 rpm/ 15 minutes. Supernatant was assayed. 1ml of supernatant and 0.5 ml of DTNB reagent + 3ml of phosphate buffer was added. Yellow colour was observed. Read at 412 nM in U.V spectrophotometer.

Chemicals

- Tris Buffer: 0.4 M p^H 7.0
- 2.42 grams of tris buffer is dissolved in 50ml of distilled water. P^H adjusted with 1N Hcl.
- Sodium azide 10mM
- 0.032 mg is dissolved in 50ml of distilled water.
- TCA 10%:
- 10 grams is dissolved in 100ml.
- EDTA: 0.4mM
- 8 grams of EDTA is dissolved in 50ml of distilled water.
- H_2O_2: 20mM

- 0.020 grams is dissolved in 30ml of distilled water. 20µl is taken from that and is dissolved in 30ml of water.
- Glutathione 2mM:
- 0.0184 grams is dissolved in 30ml of distilled water.
- Edman's reagent:
- 19.8mg of DTNB is dissolved in 100ml of 1% sodium citrate solution.

Estimation of Malondialdehyde (Lipid Peroxidation)

Procedure

The estimation of the Malondialdehyde is following the OHKWA ET. Al. 1979 which is as follows The tissue samples were homogenized in 5% 50mM phosphate buffer p^H 7.0 containing 0.1mM EDTA. The above sample is centrifuged at 10,000 rpm/ 10 minutes at 0^0 C, discard the pellet and the supernatant was used for the estimation of MDA. 200µl of tissue supernatant was added to 50µl of 80% SDS, vortexes and incubated for 10 minutes at room temperature. Add 375µl of 20% Acetic acid and 375µl of 0.6% Thiobarbituric acid and placed in a boiling water bath for 60 minutes (95^0C). Samples were allowed to cool at room temperature. Add 1.25ml of Butanol- Pyridine (15:1), vortexes and centrifuged at 4000 rpm/ 10 minutes. Take organic layer and read at 532 nm.

Chemicals

- Pottasium phosphate buffer
- 8.1% Sodium dodecyl sulphate
- 20% Acetic acid
- 0.6% Thiobarbituric acid
- 15:1 Butanol- pyridine

Estimation of Total Atpases

Procedure

The estimation of total ATPase's follows the Terri *et al*., 1973. This method is as follows. 1% homogenate of a tissue was prepared in 0.25 M ice-cold sucrose solution. 2.6 ml of reaction mixture contains: 0.5ml of Tris buffer + 0.4ml of ATP + 0.5ml of $Mgcl_2$ + 0.5ml of Nacl + 0.5 ml of Kcl + 0.2 ml of homogenate. The above contents were incubated at 37^0C for 15 minutes. Reaction was terminated by adding 1.5 ml of 10% TCA Centrifuged at 1000 rpm for about 15 minutes. 1 ml of supernatant + 1ml of Ammonium molybdate solution + 0.4 ml of ANSA (Allowed to react for 5 minutes). Blue colour was formed Read at 600nm in spectrophotometer against reagent blank.

Blank: 1.5ml of TCA + 1ml of ammonium molybdate + 0.4 ml of ANSA.

Chemicals

- 0.25 M Sucrose: 4.278 grams dissolved in 50 ml of distilled water.
- Tris buffer: 1.024 grams dissolved in 50 ml of distilled water.
- ATP: 0.110 grams in dissolved in 50 ml of distilled water.
- $Mgcl_2$: 0.508 grams is dissolved in 50 ml of distilled water.
- Nacl: 0.146 grams is dissolved in 50 ml of distilled water.
- Kcl: 0.186 grams is dissolved in 50 ml of distilled water.

Estimation of Water Soluble Proteins

Total protein content was estimated by the Lowry method. The method is having the following procedure

1% of the lens tissue was homogenized in 0.25M ice cold sucrose solution. 0.5 ml of the crude homogenate is thoroughly mixed with 1ml of Trichlor acetic acid. Then centrifuged at 1000 rpm for about 15 minutes. Discard the supernatant liquid. The pellet was dissolved in 1ml of 1N NaOH. Then 4ml of alkaline copper reagent was added followed by 0.4 ml of Folin- phenol Reagent (Folin reagent):(Distilled water){1:1}. Read the colorimeter at 600nm.

Chemicals Required

- 10% TCA: 10 grams of Trichloro acetic acid is dissolved in 100ml of distilled water.
- 1N NaOH: 4 grams of NaOH is dissolved in 100ml of distilled water.
- Alkaline copper reagent (ACR):

Solution A: 0.4 grams of NaoH was dissolved in 100ml of distilled water. Then 2 grams of Na_2Co_3 was added.

Solution B: 0.5 grams of $CuSo_4$ was dissolved in 100 ml of distilled water. Then 1 gram of sodium potassium tartarate was added.

Solution A: 50 ml
Solution B: 1ml

Both the solutions were mixed properly.

Folin Phenol reagent: These is prepared by making 1:1 dilution i.e. 1ml of folin phenol reagent: 1ml of distilled water.

RESULTS

Phytochemical Parameters

Table This table describes the results of the phytochemical parameters of the petroleum ether, ethanolic and aqueous extracts.

S.No	Phytochemicals	Petroleum Ether Extract	EthanoliC Extract	Aqueous Extract
1.	Alkaloids	++	++	++
2.	Glycosides	++	+	++
3.	Triterpinoids	+	+	+
4.	Steroids	+++	+++	++
5.	Saponins	+	+	+
6.	Flavonoids	+++	+++	+++
7.	Tannins	+	+	+
8.	Carbohydrates	Absent	Absent	Absent

\+ = Present
++ = Moderately present
+++ = Apreciable amount.

Photographic Evaluation

The photographic examination of the eyes showed that lenses of control were in normal stage throughout the duration of experimental period. Lenses treated with glucose (55mM) showed varying degree of cataractogenic changes as evidenced by opacification starting at the periphery after 8 hours. Treatment with various concentrations of petroleum ether, ethanolic extract and aqueous extracts of seeds of Syzygium cumini retarded the progression of lens opacification and this could be evidenced with clear visibility of gridlines through the lens.

Photographic Evaluation of Chick Lens

The check lens were divided into the following groups for different extracts of *Syzygium cumini* seed powder.

The petroleum ether, ethanolic, aqueous extracts of Syzygium cumini were divided into the following groups as shown above. The grouping for all the three extracts is same. Grouping of the chick lens for different extracts is as above
The lenses were placed on a wired mesh with posterior surface touching the mesh, and the pattern of mesh (number of squares clearly visible through the lens) was observed to measure the lens opacity.

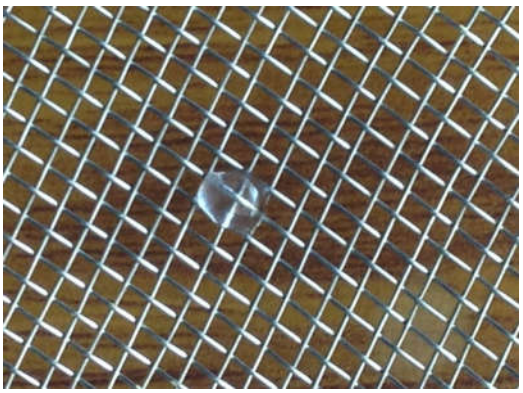
Photographic Evaluation of Chick Lens

Table This table describes the results of the photographic evaluation of the chick lens with normal control, toxic control, standard drug and different extracts.

S. No	Degree of Opacity	Normal Control		Toxic Control		Standard Drug (Enalapril)		Petroleum Ether Extract		Ethanolic Extract		Aqueous Extract	
		1st hr	8th hr	1st hr	8th hr	1st hr	8th hr	1st hr	8th hr	1st hr	8th hr	1st hr	8th hr
1.	Absence of opacity	0	0	+	+	0	0	0	0	0	0	0	0
2.	Slight degree of opacity	-	-	+	+	-	-	-	-	-	-	-	-
3.	Presence of diffuse opacity	-	-	++	++	-	-	-	-	-	-	-	-
4.	Presence of extensive thick opacity	-	-	++	+++	-	-	-	-	-	-	-	-

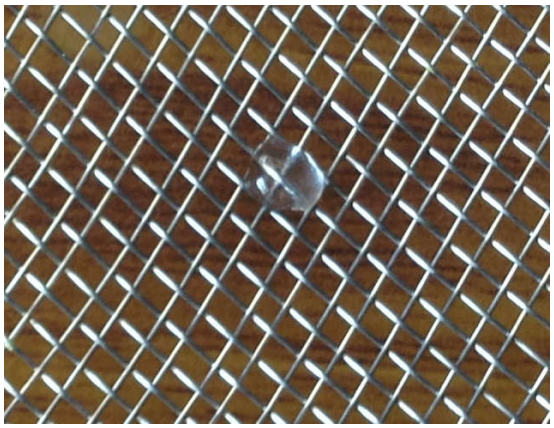
The degree of opacity was graded as follows:

1. Absence of opacity: 0
2. Slight degree of opacity: +
3. Presence of diffuse opacity: ++
4. Presence of extensive thick opacity: +++



Normal control

Toxic control



Petroleum ether extract

Aqeous extract

Ethanolic extract

Photographic evaluation of goat lens

The goat lens were divided into the following groups for different extracts of *Syzygium cumini* seed powder. The petroleum ether, ethanolic, aqueous extracts of *Syzygium cumini* were divided into the following groups as shown above. The grouping for all the three extracts is same. Grouping of the goat lens for different extracts is as above mentioned groups.

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

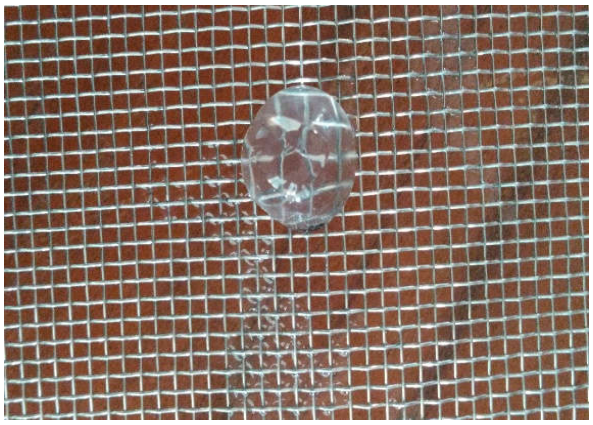
Photographic Evaluation of Goat Lens

Table This table describes the results of the photographic evaluation of the Goat lens with normal control, toxic control, standard drug and different extracts.

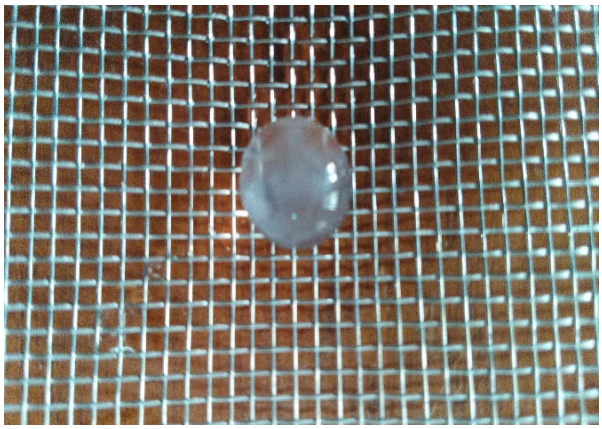
S. No	Degree Of Opacity	Normal Control		Toxic Control		Standard Drug (Enalapril)		Petroleum Ether Extract		Ethanolic Extract		Aqueous Extract	
		1st hr	8th hr	1st hr	8th hr	1st hr	8th hr	1st hr	8th hr	1st hr	8th hr	1st hr	8th hr
1.	Absence of opacity	0	0	+	+	0	0	0	0	0	0	0	0
2.	Slight degree of opacity	-	-	+	+	-	-	-	-	-	-	-	-
3.	Presence of diffuse opacity	-	-	++	++	-	-	-	-	-	-	-	-
4.	Presence of extensive thick opacity	-	-	++	+++	-	-	-	-	-	-	-	-

The degree of opacity was graded as follows:

- Absence of opacity: 0
- Slight degree of opacity: +
- Presence of diffuse opacity: ++
- Presence of extensive thick opacity: +++



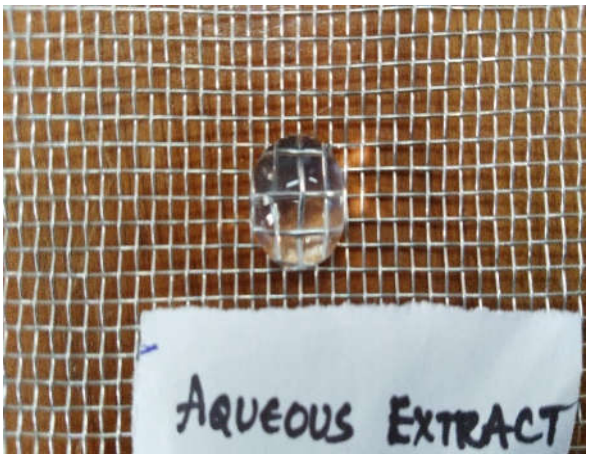
Normal control



Toxic control

Ethanolic Extract - Standard Drug

Petroleum ether extract



Aqueous extract

Treatment with various concentrations of petroleum ether, ethanolic extract and aqueous extracts of seeds of *Syzygium cumini* on chick and goat lens showed that the extract retarded the progression of lens opacification and this could be evidenced with clear visibility of gridlines through the lens.

Petroleum Ether Extracts (Mean ± S.D)

Table This table describes the values of the Total proteins, Water soluble proteins, Total ATP ases, Glutathione peroxidase, Catalase and Malondialdehyde values of the petroleum ether extract.

S.NO	Experimental Groups	Total Proteins (mg/g)	Water Soluble Proteins (mg/g)	Total ATP' ases (µg/g lens)	Glutathione Peroxidase (µmole/gm)	Catalase (µmole/gm)	Malondialdehyde (MDA) (µmole/gm)
1	Group I: Glucose5.5mM (normal control)	1.2082±0.23	1.29787±0.01	1.11175±0.01	0.97361±0.04	1.8995±0.08	2.128±0.02
2	Group II: Glucose55mM (toxic control)	1.43078±0.52	1.53053±0.50	1.23099±0.25	1.31031±0.32	2.29877±0.37	3.24262±0.57
3	Group III: Glucose 55mM+ Standard drug+ Lens culture	1.92281±0.25	1.92464±0.24	1.32338±0.05	1.39762±0.06	3.12744±0.09	2.89802±0.01
4	Group IV: Glucose55mM+Petroleum ether extract 50µg/ml+ Lens culture	1.90077±0.04	1.83541±0.03	1.49392±0.10	1.41807±0.07	3.33918±0.08	2.09467±0.02
5	Group V: Glucose55mM+Petroleum ether extract 100µg/ml+ Lens culture	2.33068±0.54	2.31418±0.035	1.60016±0.41	1.52507±0.14	3.38268±0.27	1.80284±0.08
6	Group VI: Glucose55mM+Petroleum ether extract 150µg/ml+ Lens culture	2.92014±0.716	2.95642±0.41	1.79866±0.33	1.64537±0.06	3.48111±0.07	1.94552±0.04

Aqeous Extract (Mean ± S.D)

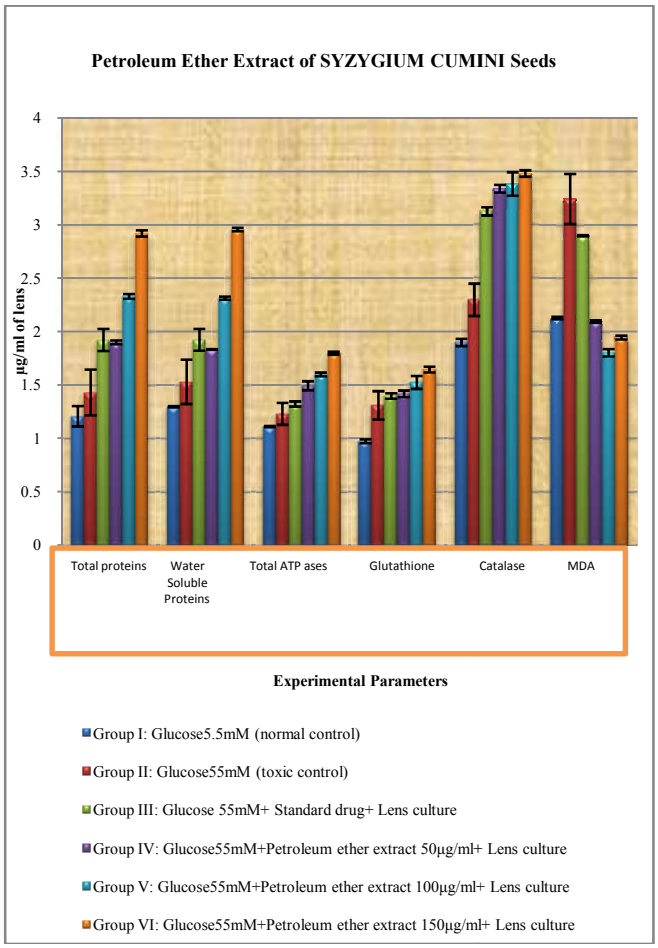
Table This table describes the values of the Total proteins, Water soluble proteins, Total ATP ases, Glutathione peroxidase, Catalase and Malondialdehyde values of the Aqueous extract.

S.NO	Experimental Groups	Total Proteins (mg/g)	Water Soluble Proteins (mg/g)	Total ATP' ases (µg/g lens)	Glutathione Peroxidase (µmole/ gm)	Catalase (µmole/gm)	Malondialdehyde (MDA) (µmole/gm)
1	Group I: Glucose5.5mM (normal control)	0.992±0.003	1.00526±0.006	0.90817±0.003	1.20687±0.008	1.03484±0.01	2.23913±0.06
2	Group II: Glucose55mM (toxic control)	1.43155±0.01	1.41661±0.006	1.02384±0.010	1.30213±0.005	2.60377±0.01	2.62266±0.04
3	Group III: Glucose 55mM+ Standard drug+ Lens culture	1.64452±0.004	1.65004±0.007	1.21256±0.005	1.29672±0.01	2.80446±0.01	1.62626±0.01
4	Group IV: Glucose55mM+Aqueous extract 50µg/ml+ Lens culture	1.62726±0.007	1.64143±0.06	2.31069±0.009	1.40409±0.006	2.89454±0.009	2.10782±0.08
5	Group V: Glucose55mM+ Aqueous extract 100µg/ml+ Lens culture	2.3247±0.10	2.3029±0.01	2.92823±0.01	1.41743±0.07	3.20791±0.01	1.62822±0.007
6	Group VI: Glucose55mM+ Aqueous extract 150µg/ml+ Lens culture	2.91364±0.11	2.82403±0.07	2.93672±0.01	1.42461±0.006	3.22771±0.008	1.43048±0.008

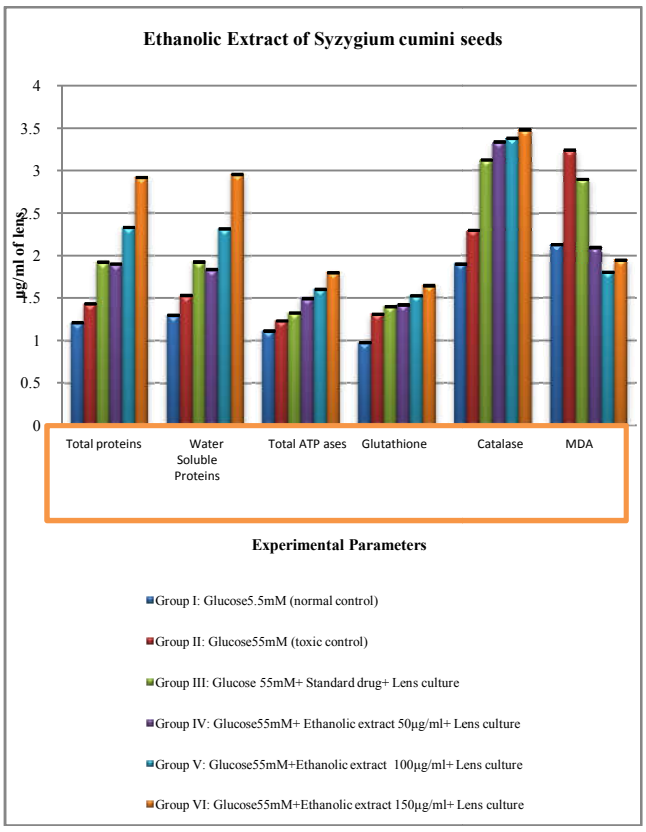
Ethanolic Extract (Mean ± S.D)

Table This table describes the values of the Total proteins, Water soluble proteins, Total ATP ases, Glutathione peroxidase, Catalase and Malondialdehyde values of the Ethanolic extract.

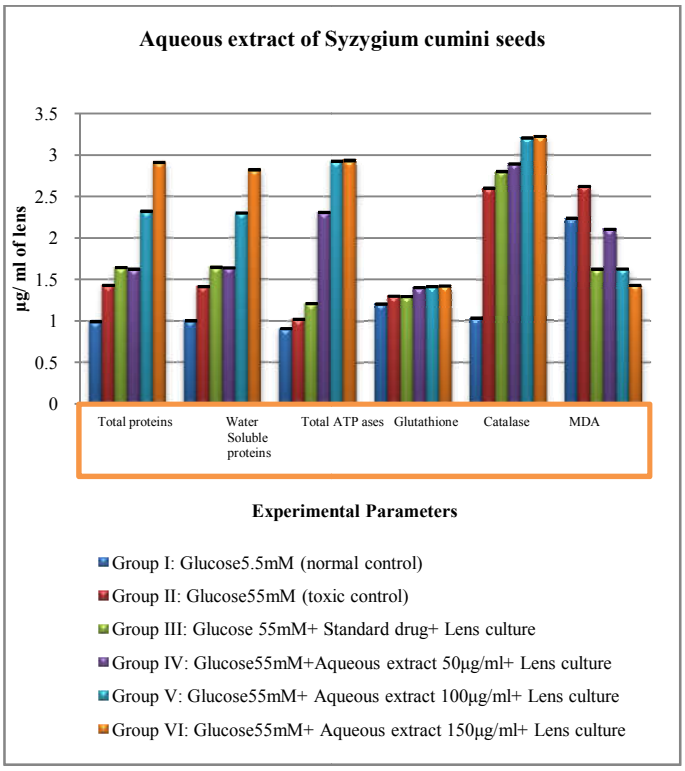
S.NO	Experimental Groups	Total Proteins (mg/g)	Water Soluble Proteins (mg/g)	Total ATP' ases (µg/g lens)	Glutathione Peroxidase (µmole/gm)	Catalase (µmole/gm)	Malondialdehyde (MDA) (µmole/gm)
1	Group I: Glucose5.5mM (normal control)	1.2082±0.01	1.29787±0.01	1.11175±0.09	0.97361±0.07	1.8995±0.10	2.128±0.005
2	Group II: Glucose55mM (toxic control)	1.43078±0.05	1.53053±0.09	1.23099±0.09	1.31031±0.008	2.29877±0.009	3.24262±0.01
3	Group III: Glucose 55mM+ Standard drug+ Lens culture	1.92281±0.08	1.92464±0.01	1.32338±0.08	1.39762±0.008	3.12744±0.005	2.89802±0.009
4	Group IV: Glucose55mM+ Ethanolic extract 50µg/ml+ Lens culture	1.90077±0.06	1.83541±0.09	1.49392±0.01	1.41807±0.014	3.33918±0.005	2.09467±0.013
5	Group V: Glucose55mM+Ethanolic extract 100µg/ml+ Lens culture	2.33068±0.04	2.31418±0.01	1.60016±0.01	1.52507±0.005	3.38268±0.01	1.80284±0.009
6	Group VI: Glucose55mM+Ethanolic extract 150µg/ml+ Lens culture	2.92014±0.57	2.95642±0.01	1.79866±0.01	1.64537±0.14	3.48111±0.01	1.94552±0.008



Graph This graph describes the values of biochemical parametrs of petroleum ether extract on different doses and comparision of the extract values with the standard drug, normal and toxic control.



Graph This graph describes the values of biochemical parametrs of Ethanolic extract on different doses and comparision of the extract values with the standard drug, normal and toxic control.



Graph This graph describes the values of biochemical parametrs of Aqueous extract on different doses and comparision of the extract values with the standard drug, normal and toxic control.

Statistical Analysis

All data were expressed as mean ± S.D. All data were analyzed with SPSS/20 (IBM) software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by multiple comparisons and homogenate variables were analyzed. All the values are expressed as mean ± S.D and results were considered significantly different if $P< 0.05$. Statistical analysis were done and the values were analyzed with POST HOC, TUKEY, SCHEFFE T3, and DUNNETT. Data was considered statistically significant at $P\leq 0.05$.

DISCUSSION

Cataract, the clouding of the lens develops later in the life and it is most likely the consequence of decades of accumulated damage to long lived lens protein. It is the most common cause of blindness. Oxidative stress is an important factor in the development of cataracts. The parameters commonly considered in cataractogenesis are total proteins, water soluble proteins, catalase levels, malondialdehyde, glutathione and total ATPase's. Incubation in the media containing high glucose (55mM) concentration has shown to cause considerable drop in Na^+-K^+- ATPase activity, with progression of opacity. Na^+-K^+- ATPase is important in maintaining the ionic equilibrium in the lens, and its impairment causes accumulation of Na^+ and loss of K^+ with hydration and swelling of the lens fibers leading to cataract formation. This alteration in the Na^+- K^+ ratio alters the protein content of the lens, leading to a decrease in water soluble protein content and increase in insoluble proteins. This cause lens opacification.

Oxidative stress is a common underlying mechanism of catarectogenesis and anti- oxidant defenses has been shown by these extract to prevent or to delay cataract. Reduced glutathione is another mechanism behind the formation of the

cataract. Hence the extract should increase the glutathione concentration. This study showed higher Na^+-K^+- ATPase activity, total and water soluble proteins, catalase levels, glutathione levels and decrease in the malondialdehyde in all the three extracts. The MDA levels were significantly higher in high glucose (55mM) groups, compared with the normal control group. The MDA levels were significantly lower in enalapril and *Syzygium cumini* seed extract treated groups. Enalapril and *Syzygium cumini* seed extract treated groups have also been shown to increase the total and water soluble proteins, retarding the process of cataractogenesis which is initiated by high glucose concentration. But when we compare the three extracts ethanolic extract is having the lower levels of malondialdehyde when compared with the standard drug.

Incubation in the presence of high glucose (55mM) concentration stimulates a state of clinical diabetes. A prevention role of *Syzygium cumini* as seen in this in vitro model may to some extent suggest in preventing or retarding the progression of diabetic cataracts. This in vitro study may not directly correlate with the in vivo conditions. Therefore in vivo studies in different animal models are required for further elucidation of the role of *Syzygium cumini* in preventing cataract formation.

CONCLUSION

The result revealed the presence of biologically active compounds and important phytochemical constituents in the petroleum ether extract, ethanol and aqueous extracts of *Syzygium cumini* seeds which may be worth for further investigation and elucidation. This research also revealed the anti- cataract activity of the *Syzygium cumini* seeds with different extracts.

This research provides the relevant information regarding the anti- cataract activity of *Syzygium cumini* seeds on goat lens and chick lens. Since eye is a unique organ, it is constantly exposed to oxidative stress and protection of lens from these is critical for essential. So far, no single compound has found wide spread acceptance for these indication, although many compounds have been screened. Thus, there is need for further research in this area. Therefore this research enables as an important segment for development of effective medicine for treatment of cataract. Hence the present investigation opened avenues for the treatment of cataract from the *Syzygium cumini* seed extract.

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