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SHORT COMMUNICATION

Sesamum indicum L. Seed Methanol Extract Inhibits Glucose-induced Cataract on Isolated Goat Eye Lens

Ujjval P. Vaghela[1*], Anand Bhalodiya[2], Harshita Mahajan[2], Divy Bhimani[2], Monika Patel[2], Sampada Kirkite[2], Kushal Parekh[2], Ashwini Dhruv[2], Krupali Makwana[2], Vishvesh Kanabar[2], Raviraj Prajapati[3] and Dilsar Gohil[1]

[1]Department of Pharmacy, Sumandeep Vidyapeeth Deemed to be University, Vadodara – 391760, Gujarat, India; ujjvalvaghela08@gmail.com

[2]School of Pharmacy, R. K. University, Rajkot- 360020, Gujarat, India

[3]School of Pharmacy, Parul University, Vadodara – 391760, Gujarat, India

Abstract

Background: Most cases of blindness and other visual impairments are caused by cataracts. Cataracts result from free radicals and oxidative stress in diabetes. Overall, antioxidants reduce cataract development. Potential antioxidants from plants abound. Polyol buildup from aldose reductase increases intracellular fluid and ocular fluid retention. Several metabolic pathways generate cataracts after swelling. 5 AMP- Activated Protein Kinase (AMPK) downregulation is linked to cataract development. Chemicals found in *Sesamum indicum* L. (*Til*) seeds contain lignans like sesamin, sesamol, and sesaminol, tocopherols like α- and γ-tocopherol, and phytosterols of β-sitosterol and stigmasterol. This phytochemical has antioxidant, anti-inflammatory, and antibacterial effects. They also inhibit aldose reductase and increase AMPK. **Aim:** *Sesamum indicum* L. seed methanol extract inhibits glucose-induced cataract in isolated goat eye lens. **Methods:** In the study, six groupings of 36 lenses were given different concentrations of *Sesamum indicum* seeds: 5.5mM control, 55mM disease, 12 ng/ml standard, 250 µg/ml extract, 500 µg/ml extract, and 1000 µg/ml extract. The evaluation parameter included lens shape and opacity photography. Total protein and catalase activity were the biological measurements. **Results:** The antioxidant action and suppression of the aldose reductase-polyol pathway attained by using the seeds extract of *Sesamum indicum* clearly showed a significant decrease in lens opacity. Compared to the disease group, there was a substantial rise in total protein and catalase enzyme activity. **Conclusion:** The methanol seed extract of *Sesamum indicum* proved antioxidant activity, regulation of the polyol pathway, and function in reducing diabetic cataract development in patients. The experiment was carried out with a goat eye lens model produced *in vitro* from glucose.

Major Finding: We found antioxidants in *Sesamum indicum* L. seeds that suppress cataract polyol pathway development. Seeds enhance total protein and catalase enzyme activity, reducing lens opacity and cataracts.

Keywords: Antioxidant, Cataract, Polyol Pathway, *Sesamum indicum*

1. Introduction

Cataract development is a serious disorder of the eye that can cause blindness. Its unique characteristics are opacification of the eye lens and lack of transparency. An opaque lens scatters light as it goes through it, therefore impairing the retina's capacity to see clear images and distorting vision[1]. The development of cataracts is connected to a wide spectrum of elements working over many years. Reactive oxygen and nitrogen species, free radicals, and other environmental elements damage the crystalline lens and lead to cataract formation[2]. This review has covered several pathways and events linked to oxidative damage from the perspective that leads to cataract development, as well as the present cataract treatments.

*Author for correspondence

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Cataracts in the lenses account for the main cause of blindness and visual difficulty worldwide. More than 68% of persons over 79 are thought to have a cataract or diminished lens opacity; the disorder is more prevalent with age. Lens cataracts can be clinically divided into four basic types: nuclear, cortical, sub-capsular, mixed (nuclear and cortical)[3,4]. Diabetic cortical cataract and Age-Related Nuclear (ARN) cataract are the two most often occurring forms of these cataracts. Rising population, inactive lifestyles, bad diets, and other factors are driving people toward diabetes mellitus at an accelerating pace. Over 285 million people have diabetes mellitus worldwide; by 2030, that figure is anticipated to rise to 439 million. Common complications of both type 1 and type 2 diabetes, and diabetic cortical cataracts, affect diabetic individuals 2-3 times more often and at a younger age. The astonishing rate at which our ageing population is growing will be shown by twice as many persons in our country who are 60 years of age or older by 2025. With ageing being a key risk factor, cataracts are thought to be around 20 million in global frequency[4,5].

2. Pathophysiology

Animal experimental research shows that polyols accumulating intracellularly result in the development of lens opacities. This causes lens fibres to break and collapse. These facts led one to create the "Osmotic Hypothesis" of sugar cataract production. It emphasises how lens oedema results from AR-mediated polyol buildup, increasing intracellular fluid. This swelling then relates to intricate metabolic processes that cause cataract development. Furthermore, investigations have revealed that osmotic stress in the lens resulting from sorbitol accumulation causes Lens Epithelial Cells (LEC) to die, which in turn promotes cataract development. Diabetic cataracts were more likely to arise in transgenic hyperglycaemic mice expressing both the Androgen Receptor (AR) and Phospholipase D (PLD) genes than in diabetic mice expressing only PLD alone, an enzyme vital to lens osmoregulation. These results imply that abnormalities in osmoregulation could expose the lens to minute increases in osmotic stress generated by AR, hence promoting the eventual development of cataracts[6,7].

The main agent of oxidative stress in the lens brought on by diabetes is the polyol pathway. The major location of protein synthesis, the Endoplasmic Reticulum (ER), accumulates sorbitol-induced osmotic stress generates free radicals. Variations in glucose levels can also induce an Unfolded Protein Response (UPR), therefore generating reactive oxygen species (ROS) and oxidative stress to lens fibres. Recent research has specifically outlined how free radical scavengers from diabetes damage lens fibres via oxidative stress. But there is no proof that these free radicals induce cataract development; rather, they just hasten and aggravate it. Aqueous humour and the diabetic lens have more hydrogen peroxide, which could generate more peroxynitrite and so harm cells as it is an oxidising agent[7,8].

The commonly known as *Til*, a Pedaliaceae family plant, has phytochemical components in several seeds. Phytosterols like β-sitosterol and stigmasterol, lignans including sesamin, sesamol, and sesaminol, and tocopherols including α-tocopherol and β-tocopherol have been discovered in this plant. These phytochemical elements combat bacteria, have anti-inflammatory and antioxidant effects[9,10]. Moreover, they can increase the activity of AMPK by lowering the activity of the aldose reductase enzyme. This work sought to evaluate, using an *in vitro* model of glucose-induced cataract in goat eye lenses, the prospective anti-cataract capabilities of *Sesamum indicum* L. seeds. Investigating lens parameters, including shape, total protein content, catalase activity, and specific antioxidant activity, the study project sought to prevent cataracts and introduce antioxidants.

3. Experimental Materials and Procedures

3.1 Collection and Verification of Plant Material

Sesamum indicum L. (*Til*) seeds were brought and gathered near Talaja in Bhavnagar city. The RK University in Rajkot, Gujarat and the Department of Botany at the School of Science authenticated the plant.

3.2 Preparation of Plant Extract

To make powder, the plant seeds were ground, and the powder was given a 48-hour methanol maceration. After being dried out by evaporation at 40°C, the extracts were kept at 4°C for storage. The assessment of

phytochemical elements such as flavonoids, glycosides, and phytosterol compounds was assessed as previously described

3.3 Chemicals and Reagents

The glucose was procured from the ASTRON lab (located in India). AUTOSPAN® provided the total Protein and Catalase kits. The chemicals utilised were all of the analytical grade.

3.4 Collection of Goat Eye and Lens Culture

Goat eyes were delivered fresh from Sadar Bazaar and the slaughterhouse in Vadodara. Immediately after death, the eyes were removed by the butcher and kept between 0 and 4 degrees Celsius. The lenses were extracted extracapsularly, and artificial aqueous humour was cultured from them. 140 mM NaCl, 5 mM KCl, 22 mM MgCl, 0.5 mM NaHCO3, 20.5 mM NaH(PO4), 0.4 mM CaCl2, and 5.5 mM glucose were combined with a pH 7.8 solution for 72 hours. Penicillin 32 mg% and streptomycin 250 mg% added to prevent bacterial contamination of the cultural media[11,12].

3.5 Generating the Glucose-Induced Cataract Model

Using a glucose concentration of 55mM, cataracts were induced. As the lens's high glucose contents were processed by the sorbitol route, polyols, also known as sugar alcohols, accumulated. This produced oxidative stress and dried the lens. Cataractogenesis emerged from this. The extract was diluted with Dimethyl Sulfoxide (DMSO) to carry out the experiment[11,13].

3.6 Groups

The following six categories were established using a total of six lenses (n=6):

Group I: A glucose level of 5.5mM was considered a Normal control group

Group III: 55mM glucose -Disease group

Group II: 55mM glucose plus 12 ng/ml enalapril

Group IV: 55mM glucose plus 250 µg/ml seed extract of *Sesamum indicum*

Group V: 55mM glucose plus 500 µg/ml seed extract of *Sesamum indicum*

Group VI: 55mM glucose plus 1000 µg/ml seed extract of *Sesamum indicum*

3.7 Studying an Image's Lens Opacity

To find the lenses' opacity, the posterior sides of the lenses were set on a piece of graph paper and incubated for a whole day. The following criteria helped to evaluate the opacity degree[11-13]:

0 indicates no opacity,

+ indicates a small amount of opacity.

++ indicates diffuse opacity

++++ indicates a significant amount of thick opacity,

3.8 Creating the Lens Homogenate

Ten per cent of every lens was homogenised in a sodium phosphate buffer with a pH of 7.4 and a concentration of 0.1 M following 72 hrs incubation period. After that, the homogenate was centrifuged for 30 minutes at 10,000 grams and -4 degree Celsius. The supernatant was stored for additional study at -20 degrees celsius[13].

3.9 Biological Parameter

Estimated from the supernatant, the biochemical parameters include total protein and catalase activity. The Biuret technique allowed one to determine the total protein composition of the lens. Catalase activity level was assessed by Ultraviolet (UV) spectrophotometry assays.

3.10 Statistical Analysis

GraphPad Prism 9 was used as a tool in the statistical analysis, applying a one-way Analysis of Variance (ANOVA). The mean ± SEM (standard error of the mean) is shown for every set of six-lens results. P values falling below 0.05 were considered statistically significant.

4. Results

People between the ages of 45 and 50 are most often affected by cataracts, which cause lenses to be opaque due to oxidative stress. In replacement and removal of clouded lenses, intraocular lenses have a higher success rate than in previous treatments. Rarely, surgical intervention could cause endophthalmitis, also known as vision loss[14,15]. Investigating non-invasive approaches to reverse the early phases of cataract, including nutraceuticals or herbal extracts, is thus

rather important. Aiming to investigate the antioxidant and anticatalytic activities of a methanol extract of *Sesamum indicum* seeds, this work uses glucose-induced cataracts in goats as a model.

4.1 Assessment of Lens Opacities by Photograph

Photographs of normal and experimental lenses were assessed with a grid mesh following 72 hours of incubation. Compared to other lenses, complete opacity in the Group II (disease) incubated lenses maintained in 55mM glucose was noted (Figure 1 and Table 1). The opacity in the lenses moved from outward to inward toward the conclusion of the 72 hours. Group IV showed the highest clarity when the lenses were incubated with the methanol extract of *Sesamum indicum* L. seeds; Group V and VI followed (Table 1 and Figure 1). The clarity of the lenses observed determines just how effective the extract is. Lenses cultured in the usual medication, 12ng Enalapril with glucose, did not show any opacity.

4.2 Total Protein Level

Lenses devoid of cataract formation were found to have higher protein levels. Group II, lacking the sample extract, had demonstrated strong opacity. The total

Table 1. Lens opacities: photographic evaluation – degree of opacity (n=6)

Sr. No.	Treatment with *Sesamum indicum* Seeds Extract	Degree of Opacity
1	Group I: A normal control glucose level is 5.5 mM.	0
2	Group II: A disease control group is 55 mM glucose	++++
3	Group III: 55 mM glucose plus 12 ng/ml enalapril	0
4	Group IV: 55 mM glucose plus 250 µg/ml seeds extract of *Sesamum indicum*	+
5	Group V: 55 mM glucose plus 500 µg/ml seeds extract of *Sesamum indicum*	0
6	Group VI: 55 mM glucose plus 1000 µg/ml seeds extract of *Sesamum indicum*	0

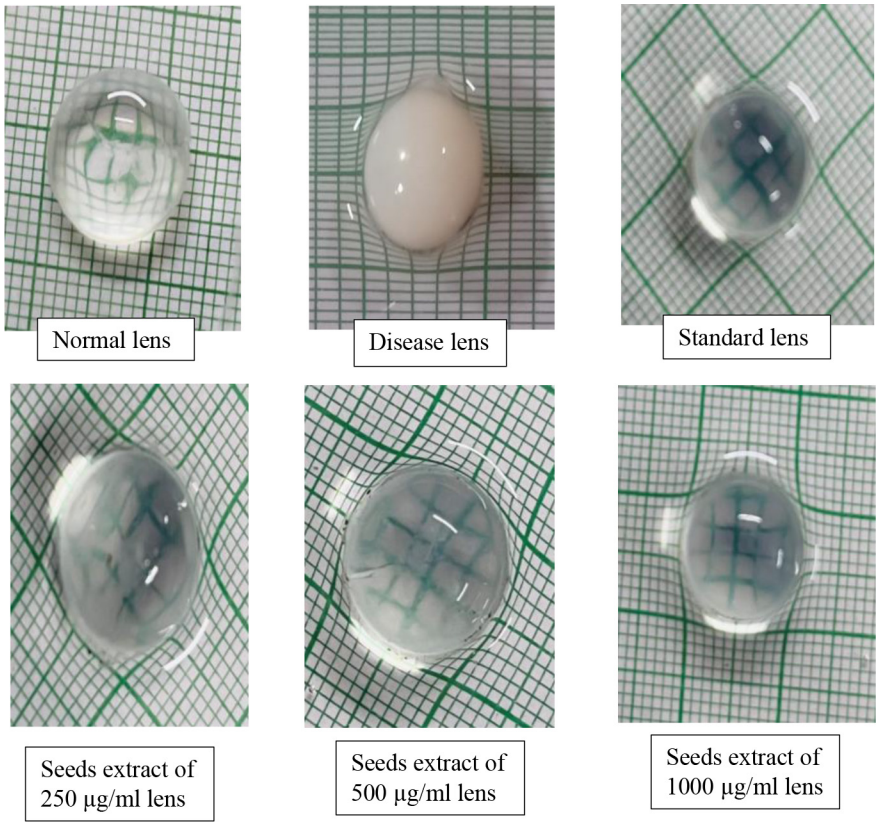


Figure 1. Photographical assessment of lens opacity following *Sesamum indicum* L. seed incubation for 72 hrs. Normal control 5.5mM Glucose; Disease Group 55mM Glucose,55mM Glucose plus + 12ng Enalapril, 55mM Glucose plus + 250 µg/ml extract of *S. indicum* L. seeds, 55mM Glucose plus + 500 µg/ml extract of *S. indicum* L. seeds,55 mM Glucose plus + 1000 µg/ml extract of *S. indicum* L. seeds.

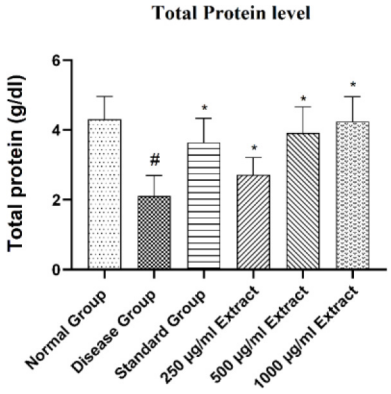


Figure 2. Group II had noticeably lower protein levels than Group I. When treated with Enalpril 12 ng/ml (Group III), *Sesamum indicum* extract treatment (Groups IV, V, VI) notably raised total protein levels over Group II. P= 0.05. With n=6, the mean and SEM display all values.

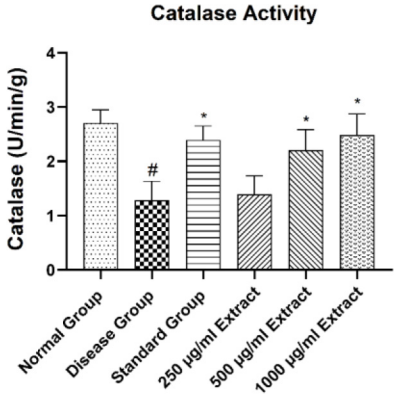


Figure 3. Group II had noticeably decreased catalase activity levels than Group I. When treated with Enalpril 12 ng/ml (Group III), *Sesamum indicum* extract treatment (Groups IV, V, VI) notably raised total protein levels over Group II. P= 0.05. With n=6, the mean and SEM display all values.

protein levels have demonstrated a notable rise in the lenses treated with the extracts. While in Groups IV, V, and VI of seeds extract of *Sesamum indicum* was 2.71* ± 0.22, 3.91* ± 0.33, 4.24* ± 0.31 g/dl, total protein levels detected in Group II was determined to be 2.10# ± 0.26 g/dl.

4.3 Catalase Activity Level

Catalase activity was higher in lenses free of cataract development. Group II's opaque sample extract-free extract-treated lenses raised catalase levels. While Group II had 1.28# ± 0.15 U/min/g, Group IV, V, and VI, *Sesamum indicum* seed extract had catalase activity levels of 1.39* ± 0.15, 2.20* ± 0.16, 2.48* ± 0.17 U/min/g.

5. Discussion

Ayurvedic literature has long noted the antidiabetic qualities of the *Sesamum indicum* L. seeds[16]. But it was necessary to evaluate how these plant extracts affected the oxidative stress-related biochemical changes occurring in cataract, as well as the lens shape[17]. The present work developed an *in vitro* cataract model in goat lenses using 55mM of glucose. It is well recognised that oxidative stress contributes to the development of diabetes and its consequences, including cataract. High concentration dextrose causes the glucose in the lens to be used via a sorbitol route. Overhydration and oxidative stress brought on by the accumulation of polyols—sugar alcohols cause cataract development. Different channels of oxidative stress are caused by hyperglycaemia. Insoluble protein aggregates are produced from further oxidation of the lens crystalline as well as membrane proteins. The present study shows that loss of soluble proteins from the lens by their conversion into insoluble ones is reflected as a decrease in total lens protein content due to lens protein oxidation[18-20].

Various criteria, including lens shape, contour, swelling, and transparency, were used to investigate the goat lens morphology, and the degree of opacity graded the cataract modifications. Grade greater opacity in the goat lenses was observed when 55mM glucose was indicated (Figure 1). Lens shape was preserved against cataract development and opacity of *Sesamum indicum* L. by the use of seed extract. The results of the present investigation support those of previous studies. Raised oxidative stress compromises the enzymatic protection against reactive oxygen species. This is shown by the notable drop in several antioxidant enzymes in cataract in experimental models of lenses. Phytochemical antioxidants, including sesamin, sesamol, and sesaminol as well as tocopherols, including α-tocopherol, γ-tocopherol and phytosterols made of β-sitosterol and stigmasterol. *Sesamum indicum* methanol seed extract added showed notable antioxidant activity (Figures 2 and 3) by changes in the total protein level and catalase activity level. Richness of phytochemical antioxidants in *Sesamum indicum* plant extracts guides to their likely function in the prevention of lipid peroxidation

and therefore cataract development. The present work clearly shows the seeds of *Sesamum indicum* methanol extract by the considerable increase in activity of the antioxidant lens enzymes.

6. Conclusion

In isolated goat lenses treated with glucose to generate a diabetic cataract in an experimental model, seeds of *Sesamum indicum* extract exhibited significant antioxidant and anti-cataract properties. Still under investigation is a possible treatment using the *Sesamum indicum* L. plant seeds to prevent diabetic cataracts. Further study, including many *in vivo* animal models, is required to increase knowledge in this field.

7. Author Contribution

UV and DG conceived design, experiments, and demonstrate structure. AB, HM, DB, MP, SK, KP, AD, KM, VK, RP analyzed parameters and composed the manuscript, which UV, DG, KP, VK, RP reviewed and edited. All authors have read and agreed to the published version of the manuscript.

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