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SHORT COMMUNICATION

Anticataract Effects of Methanol Extract of *Ocimum sanctum* L. Leaf on *In Vitro* Glucose-induced Goat Eye Lens Model

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Abstract

Background: The leading cause of blindness and visual impairment is cataracts. Diabetes mellitus induces cataracts due to free radicals and oxidative stress. Antioxidants are known to slow the cataract progression. Plant-based antioxidants have great potential. Aldose reductase-mediated polyol buildup results in lens oedema due to intracellular fluid rise. After swelling, various metabolic processes cause cataracts. Downregulation of AMPK is linked to cataract formation. Thus, quercetin, luteolin, and apigenin in *Ocimum sanctum* L. leaves have antioxidant properties that block aldose reductase enzymes and regulate the AMPK. **Aim:** Anticataract effects of methanolic extract of *Ocimum sanctum* L. leaf on *in vitro* glucose-induced goat eye lens model. **Methods:** Thirty-six lenses were split among six groups (n=6). Group –I Normal control group (5.5.5 mM), Group –II Disease group (55 mM), Group – III Standard group (Enalapril 12 ng/ml), Group – IV 250 µg/ml extract of *O. sanctum* leaf, Group – V 500 µg/ml extract of *O. sanctum* leaf, Group – VI 1000 µg/ml extract of *O. sanctum* leaf. The lens morphology and photography of the lens opacity level were examined in the evaluation. The biochemical parameter of the total protein and catalase enzyme activity was measured. **Results:** The antioxidant activity and inhibition of aldose reductase-polyol pathway through the leaf extract of *O. sanctum* showed a significant reduction of lens opacity level. Also, there were significant increase in total protein in treatment groups of IV, V, VI, 250µg/ml, 500µg/ml, 1000µg/ml (g/dl), and catalase enzyme activity level in (U/min/g), as compare to disease group respectively. **Conclusion:** The methanolic leaf extract of *O. sanctum* has antioxidant activity which inhibits the polyol pathway effect on *in-vitro* glucose-induced goat eye lens model of anticataract action, and prevents diabetes cataract formation.

Major Findings: We discovered that *Ocimum sanctum* L. leaves have antioxidant properties and inhibit the polyol pathway in cataract development. The leaves also increase total protein and catalase enzyme activity, which reduces lens opacity and prevents cataracts.

Keywords: Antioxidant, Flavonoids, *Ocimum sanctum,* Polyol Pathways

1. Introduction

The pathologic state known as a cataract result from opacification of the eye's lens, which leads to vision change, colour shifts, blurriness, light-halo effects and finally, blindness. The word "cataract" comes from the Latin word "*cataracta*," which means "waterfall". A cataract results from a change in the transparency of the lens, therefore impairing vision. Cataract is the main cause of blindness and visual problems all around. With the disorder increasing prevalence as individuals age, over 68% of those over 79 are thought to have a cataract or diminished lens opacity[1].

Globally, cataract is one of the most common causes of blindness. Apart from having a 3–5 times

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higher prevalence than the healthy population, cataracts also affect younger age individuals in the diabetes community. Cataracts develop in persons with type 1 diabetes on average twenty years earlier than in the non-diabetic population[2]. Furthermore, poor metabolic control and diabetes raise the likelihood of cataracts. Lens cataracts are clinically divided into four primary forms: sub-capsular, cortical, mixed (nuclear and cortical), and nuclear. Age-Related Nuclear (ARN) cataract and diabetic cortical cataract are the two most often occurring types of cataracts. Factors like population increase, inactive lifestyles, poor diets, and increasing obesity rates are causing diabetes mellitus in more and more individuals[3,4]. Diabetes mellitus affects around 285 million individuals globally today. By 2030, this figure is predicted to rise to 439 million. Common complications of both type 1 and type 2 diabetes, diabetic cortical cataracts, affect diabetic people 2-3 times more often and at an earlier age. The significant increase in our ageing population is highlighted by the projection that, by 2025, the number of individuals aged 60 years and older in our country will have reached twice its current figure. With age being a key risk factor, the estimated worldwide prevalence of cataracts is approximately 20 million[5].

The polyol pathway is the primary arbiter of oxidative stress in the diabetic lens. Osmotic stress driven by sorbitol accumulation in the Endoplasmic Reticulum (ER), the main site of protein production, generates free radicals. Variations in glucose levels can also cause an Unfolded Protein Response (UPR), which generates Reactive Oxygen Species (ROS) and oxidative stress damages lens fibres[6].

Many recent studies have shown that oxidative stress damages free radical scavengers inflicted on lens fibres in diabetics. Though they seem to expedite and aggravate the illness, there is no evidence that these free radicals produce cataracts. Through processes known as Fenton reactions, hydroxyl radicals (OH^-) are generated, hyperglycaemia increases hydrogen peroxide (H_2O_2) in the aqueous humour, which then passes into the lens. The free radical nitric oxide (NO), also raised in the aqueous humour and the diabetic lens, causes cell damage and may aggravate the generation of peroxynitrite by its oxidising properties[7].

Furthermore, high glucose levels in the aqueous humour can lead to glycemic changes in lens proteins that generate Advanced Glycation End products (AGE) and release superoxide radicals (O^{2-}). Additional O_2 and H_2O_2 are produced when AGE and cell surface receptors, such as those for advanced glycation end products, in the epithelium of the lens interact. Apart from their higher free radical concentration, diabetic lenses have a decreased antioxidant capacity, which increases their sensitivity to oxidative stress[8]. Glycated and inactive, superoxide dismutase and other lens antioxidant enzymes aggravate the loss of antioxidants. Several investigations support the hypothesis that diabetic cataracts arise first from the synthesis of polyols from glucose by Aldose Reductase (AR). The osmotic stress this process generates in the lens fibres causes them to expand and break[9].

The development of diabetic cataract AR is an enzyme that breaks down the polyol pathway, therefore transforming glucose to sorbitol. Sugar cataracts arise from osmotic changes brought about by sorbitol's internal build-up, causing hydropic lens fibres to deteriorate. More rapidly, it turns to fructose, and the enzyme sorbitol dehydrogenase generates sorbitol in the lens. Moreover, the polarity of sorbitol prevents diffusion-based intracellular elimination. The hyperosmotic action of the rising sorbitol buildup balances the osmotic gradient using fluid. Studies on animals have shown that lens fibres break and liquefy under intracellular polyol buildup, which then generates lens opacities. These data have led to the "Osmotic Hypothesis" of sugar cataract development, which underlines that lens swelling in response to AR-mediated polyol accumulation causes lens swelling, which is subsequently connected to complex biochemical changes that finally lead to cataract development[9,10].

The antioxidant-rich leaves of the *Tulsi* plant (*Ocimum sanctum* L., Family: *Lamiaceae*) abound in phytochemicals like eugenol, rosmarinic acid, apigenin, myretenal, luteolin, β-sitosterol, and carnosic acid. The important phytochemicals eugenol, rosmarinic acid, apigenin, and carnosic acid also show protection of DNA from radiation damage. *O. sanctum* L. leaves have several medicinal uses, including anti-inflammatory, analgesic, antidiabetic, hepatoprotective, hypolipidemic, antistress, and immunomodulating[11,12].

The prospective anti-cataract properties of the *O. sanctum* plant's leaf were assessed in this work using an *in-vitro* model of glucose-induced cataract in goat eye lenses. Examining the lens in great detail and dissecting it into its total protein content, catalase activity, and particular antioxidant activity, the emphasis of the study was cataract prevention and antioxidants.

2. Methods and Materials Used in Experimentation

2.1 Collection and Verification of Plant Material

Ocimum sanctum L. leaves were collected from Vadodara district near the village of Kunvarpura. The Department of Botany, School of Science at RK University handled the plant's authentication.

2.2 Preparation of Plant Extract

The plant leaves were air-dried at room temperature to produce powder. The powder underwent 48-hour methanol maceration later. The extracts were stored at 4°C after being dried at 40°C dried. As before said, the evaluation of phytochemical components, including glycosides, phytosterol compounds, and flavonoids, was done[13].

2.3 Chemicals and Reagents

The Glucose was purchased from the ASTRON lab (India). AUTOSPAN® was the vendor of the Total Protein and Catalase kits. Every chemical utilised came in analytical grade.

2.4 Collection of Goat Eye and Lens Culture

Fresh goat eyeballs were collected from the slaughterhouse in Vadodara and the Sadar Bazaar. The abattoir removed the eyeballs when the animals died and kept them between 0 and 4 degrees Celsius. Extracapsular lenses were removed, then grown in synthetic aqueous humour. Combined for 72 hours was a pH 7.8 solution, including 140 mM NaCl, 5 mM KCl, 22 mM MgCl, 0.5 mM $NaHCO_3$, 20.5 mM NaH (PO_4), 0.4 mM $CaCl_2$, and 5.5 mM glucose. Penicillin at 32 milligrams and streptomycin at 250 milligrams were added to the culture media to prevent bacterial contamination[13,14].

2.5 Generating the Glucose-induced Cataract Model

Using 55mM glucose, we induced cataracts. The high amounts of glucose inside the lens caused it to become dehydrated and experience oxidative stress; the sorbitol pathway broke down these molecules and produced polyols, or sugar alcohols. This helped cataract genesis to evolve. The experiment was carried out with DMSO-diluted extract[13].

2.6 Group Design

Six lenses in all were utilised to generate the following six categories:

Group I: A normal control glucose level is 5.5mM.

Group II: A disease control group is 55mM glucose,

Group III: 55mM glucose plus 12 ng/ml Enalapril

Group IV: 55mM glucose plus 250 µg/ml leaf extract of *O. sanctum*

Group V: 55mM glucose plus 500 µg/ml leaf extract of *O. sanctum*

Group VI: 55mM glucose plus 1000 µg/ml leaf extract of *O. sanctum*

2.7 Studying an Image's Lens Opacity

To determine the lens' opacity, the posterior sides of the lenses were set on a sheet of graph paper and left incubated for a whole day. The following standards helped to evaluate the opacity degree[13,14]:

0 indicates no opacity,

+ indicates a small amount of opacity.

++ indicates diffuse opacity

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

2.8 Creating the Lens Homogenate

10% of every lens was homogenised using a pH 7.4 0.1 M sodium phosphate buffer after 72-hour incubation. The homogenate was then refrigerated and centrifuged for thirty minutes at 10,000 g and -4°C. The supernatant was then gathered and refrigerated at -20°C to enable the next experiments[13,15].

2.9 Biological Parameter

Among the biochemical measurements for the supernatant were total proteins and catalase activity. The total protein content of the lens was determined using the Biuret technique. The degree of catalase activity was

assessed using Ultraviolet (UV) spectrophotometry assays.

2.10 Statistical Analysis

Graph Pad Prism 9 was the instrument utilised in this statistical study, applying a one-way analysis of variance (ANOVA). Every set of six-lens results gives the mean ± SEM considered statistically significant were P values less than 0.05.

3. Results

Human ophthalmology has thus also come to see the importance of antioxidants in cataract prevention since oxidative stress has been identified as a factor in cataract development. Oxidative stress affects proteins, lipids, and DNA; cataracts are well documented to alter all three of these[15]. Three molecular events that might contribute to the start of diabetic cataract are oxidative stress, an active polyol pathway for the elimination of glucose, and a lack of enzymatic glycation of eye lens proteins. In diabetic patients' lenses, all of these changes increase the levels of oxidative chemical changes of proteins, DNA, and lipids, as well as speed up the generation of ROS[16,17]. We investigated the antioxidative and anticataract properties of *O. sanctum* L. leaf by use of an *in vitro* model of glucose-induced goat eye lens damage.

3.1 Lens Opacities Evaluated Using a Photograph

Graph paper was used to examine images taken with both regular and experimental lenses following a 72-hour incubation period. Complete opacity was seen in Group II (toxic control) lenses immersed in 55mM glucose by comparison with Normal group and treatment group lenses (Figure 1 and Table 1). The lens's opacity had shifted from an outward to an inward orientation by the end of the 72-hour timeframe. When the lenses were incubated with a methanolic extract of *O. sanctum* leaves, the clarity of the lenses observed directly corresponds to the leaf extract efficacy, best clarity was noticed in Group IV, next in Group V, and finally in Group VI. Lens development was not induced by the typical group III – Enalapril at a concentration of 12 ng/ml with glucose (Table 1 and Figure 1).

3.2 Total Protein Level

The protein level rises in lenses belonging to Groups I and III, which do not develop cataracts. Group II showed thick opacity and lowered protein levels without the *O. sanctum* extract (Fig. 1). Total protein levels were observed to be much greater in the illness group when compared to the treated lenses (Fig. 2). Measuring Group II's observed total protein levels, they came out to be (2.50 ± 0.23g/dl). The total protein levels were notably higher in Group IV, Group V, and Group VI, treated with different amounts of *O. sanctum* extract—2.59 ± 0.15* g/dl, 4.02 ± 0.25* g/dl, and 4.61 ± 0.17* g/dl, respectively. These values show a clear rise over the group whose induced sickness was based on glucose.

3.3 Catalase Activity

The Catalase activity was seen to rise in Group I lenses—lenses free of cataract development. Group II, devoid of the sample extract, showed thick opacity and decreased Catalase activity. Treatment with the extracts on lenses produced a much higher degree of catalase activity than that of the disease group (Figure 3). While in treatment of Group IV (1.20 ± 0.06*), Group V (1.85 ± 0.13*), Group VI (2.21 ± 0.08*) (U/min/g), Catalase activity levels recorded in Group II were determined to be (0.89 ± 0.09#).

Table 1. Lens opacities: Photographic evaluation – Degree of opacity (n=6)

Sr. No.	Treatment with *Ocimum sanctum* leaf extract	Degree of opacity
1	Group I: A normal control glucose level is 5.5mM.	0
2	Group II: A disease control glucose level is 55mM	++++
3	Group III: 55mM glucose plus 12 ng/ml Enalapril	0
4	Group IV: 55mM glucose plus 250 µg/ml leaf extract of *O. sanctum*	+
5	Group V: 55mM glucose plus 500 µg/ml leaf extract of *O. sanctum*	0
6	Group VI: 55mM glucose plus 1000 µg/ml leaf extract of *O. sanctum*	0

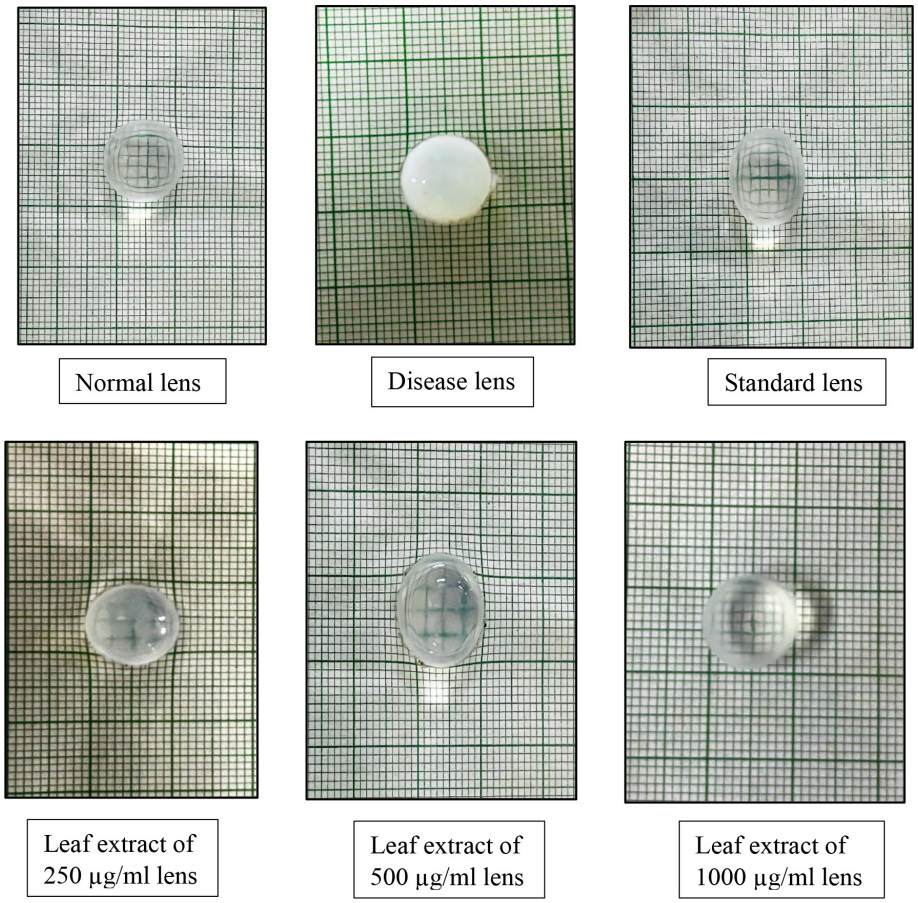


Figure 1. Evaluation of lens cloudiness using images taken 72 hours after an extract from *O. sanctum* leaves has been incubated. Group I's solution has 5.5mM glucose. (Group II) comprises just 55mM glucose. Together with 55mM glucose, Group IV includes 250 µg/ml extract of *O. sanctum*. 500 µg/ml extract of *O. sanctum* with 55mM glucose makes up Group V). Group VI calls for 55mM glucose together with 1000 µg/ml *O. sanctum* extract. Finally, (Group II) carries 12 ng/ml Enalapril in addition to 55mM glucose.

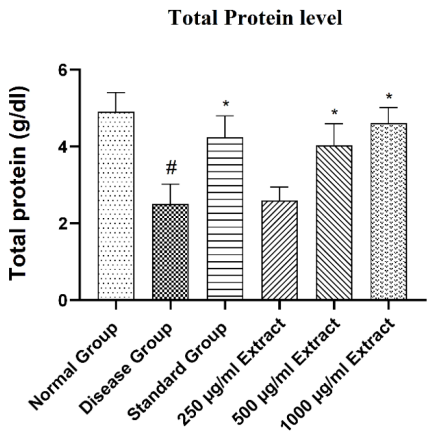


Figure 2. Group II's total protein level revealed a notable drop over Group I. Whereas that in treated with Enalpril 12 ng/ml (Group –III), *O. sanctum* extract treated (Group –IV, V, VI) was considerably raised as compared to (Group –II) total protein level. ($P < 0.05$) All values—n=6 are shown using mean and SEM.

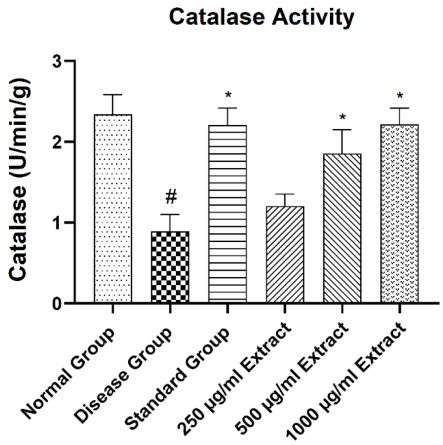


Figure 3. The catalase activity level in Group II showed a clear drop from Group I. While this in treated with Enalpril 12 ng/ml (Group –III), *O. sanctum* extract treated (Group –IV, V, VI) was considerably greater than (Group –II) catalase activity level. ($P < 0.05$). Mean and SEM display all data (n=6).

4. Discussion

Many studies have linked hyperglycemia to the decline in lens clarity. A patient with hyperglycemic problems rapidly lowers their serum glucose level, which may induce lens opacification and transient oedema. Although cataract surgery has seen many advancements, those with diabetes might not benefit as much. Among the negative effects noted are retinopathy, vitreous haemorrhage, iris neovascularisation, and either partial or total loss of vision[18,19]. Oxidative stress resulting from the generation of free radicals in the lens environment produces age-related cataracts. Apart from hydrogen peroxide and hydroxyl radicals, these free radicals also include lipid hydroperoxides and superoxide anion. Naturally high in antioxidants, the young lens contains glutathione, vitamin C and E, carotenoids, and superoxide dismutase, which might help prevent damage. It also features other antioxidant enzymes, catalase, and glutathione peroxidase[20].

One of the main risk factors for cataractogenesis, which is brought on by too high glucose levels in the blood and the generation of free radicals such as superoxide ion (O^{2-}) and H_2O_2, is the loss of the antioxidant systems of the lens. High glucose levels are well-known to activate antioxidant enzymes, which indicates oxidative stress in the cell[21,22]. When compared to a normal lens (Group I) with 5.5mM of glucose, the lens culture employed in the present experiment showed a lens total protein, catalase activity level that was markedly lower. In Group B, the lens culture treated with Enalapril and varying concentrations of *O. sanctum* leaves extract showed noticeably higher levels of total protein, catalase activity; among the groups treated with the extract, antioxidant activities increased. This helped to explain the delay of the cataractogenesis process started by high glucose concentration.

5. Conclusion

In goat lenses separated and produced with diabetes cataract using glucose, a leaf extract obtained from *O. sanctum* has shown notable antioxidant and anticataract activities. Still under active study is the possibility of using *O. sanctum* leaves to prevent diabetic cataract. Expanding our knowledge in this sector also depends on more research incorporating several *in vivo* animal models.

6. Author Contribution

UV and DG were responsible for the idea about design, experimentation, and manuscript structure. DV, BV, AR, DC, KH, KP, VK, PV analysed parameters and drafted the manuscript, which UV, DG, VK, PV was evaluated and final edited. The Final manuscript was read and approved by all authors.

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