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SHORT COMMUNICATION

Methanol Extract of *Camellia sinensis* L. Prevents *In Vitro* Glucose-induced Cataract on Isolated Goat Eye Lens

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Abstract

Background: The leading cause of blindness and visual impairment is cataracts. Diabetes causes cataracts owing to free radicals and oxidative stress. Most say antioxidants delay cataract development. Plant-based antioxidants are promising. Aldose reductase-mediated polyol accumulation causes intracellular fluid and lens oedema. Various metabolic pathways generate cataracts after swelling. Downregulation of AMPK is connected to cataracts. Tea plant leaves include antioxidant flavonoids catechin, epicatechin, gallocatechin, and epigallocatechin, which block aldose reductase enzymes and upregulate AMPK. **Aim:** Methanol extract of *Camellia sinensis* L. prevents *in vitro* glucose-induced cataract in isolated goat eye lens. **Methods:** The thirty-six lenses were evaluated in six groups (n=6). Group I had a 5.5 mM normal control, Group II a 55 mM disease, Group III a 12 ng/ml standard, Group IV 250 µg/ml *Camellia sinensis* leaves, Group V 500 µg/ml, and Group VI 1000 µg/ml. The evaluation parameter includes lens morphology and opacity photography. Biochemical parameters of total protein and catalase activity were measured. **Results:** Significant reduction in lens opacity was observed as a result of the antioxidant activity and inhibition of the aldose reductase-polyol pathway that was achieved by the use of the leaf extract of *Camellia sinensis*. A considerable increase in the level of total protein 250, 500, 1000 µg/ml (3.13 ± 0.39*), (4.19 ± 0.28*), (4.40 ± 0.37*) and catalase enzyme activity (1.83 ± 0.21*), (2.27 ± 0.19*), (2.65 ± 0.14*) was observed in comparison to the disease group. **Conclusion:** Antioxidant activity, suppression of the polyol pathway, and function in preventing diabetic cataract formation in patients were all demonstrated by the methanol leaf extract of *Camellia sinensis*. The experiment was conducted using an *in vitro* glucose-induced goat eye lens model.

Keywords: Adenosine Monophosphate Kinase, Antioxidants, *Camellia sinensis,* Polyol Pathway

1. Introduction

Cataract is the older Latin form of the term that meant "waterfall". Features of a cataract, an aberration of the lens, are increased cloudiness and reduced transparency. Cataracts are among the main worldwide causes of reversible vision loss and permanent blindness[1]. Myopia is typified by the opacification and lack of transparency of the eye lens. Because it scatters light as it goes through, an opaque lens alters vision and lowers the retina's interpretability of clear images. Problems with development, traumas, metabolic diseases, inheritance, ageing, drug-induced changes, and so on can all cause cataracts[2].

Population ageing and lifestyle changes are expected to raise the frequency of cataract; it

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already affects over 13 million persons worldwide and is the main cause of visual impairment. Lens surfaces and their light-reflecting characteristics are attributed to crystalline proteins[3]. Crystalline change, aggregation, and precipitation are quite important during the development of cataracts. We do not know at this moment how to stop this operation from happening. Although the most often occurring cause of cataracts is age-related degeneration, trauma or another illness can sometimes set off their formation. Environmental factors largely responsible for cataracts include free radicals, reactive oxygen and nitrogen species, and other stressors damaging the crystalline lens[4].

At normal glucose levels, the enzyme AR usually transforms aldehydes into alcohols, but it uses the polyol pathway to convert sorbitol from an excess amount of glucose. Using NADPH, the conversion of glucose into sorbitol causes the $NADPH/NADP^+$ ratio to drop. In this procedure, NADPH is used as a hydrogen generator. When Sorbitol Dehydrogenase (SD) oxidises sorbitol to fructose using NAD^+ as a cofactor, the ratio of NADH to NAD^+ rises. Once inside, sorbitol can upset osmotic balance and cause cell damage by challenging cell membranes. One long-standing hypothesis in acute diabetic cataract models is the intralenticular polyol accumulation brought on by hyperglycaemia[5,6].

Oxidative stress results from the discrepancy between free radical generation and the body's antioxidant defences. This tension is among the key factors causing diabetic complications. In the first phases of diabetes, oxidative stress destroys the eye lens. Consequently, important antioxidants such as reduced Glutathione (GSH) are depleted, and harmful compounds such as malondialdehyde and 4-hydroxynonenal (byproducts of lipid peroxidation) build up. Two other antioxidants, ascorbate and taurine, are reduced; the oxidised Glutathione (GSSG) to Glutathione (GSH) ratio increases[6]. The body turns up the activity of antioxidant enzymes such as glutathione peroxidase and Superoxide Dismutase (SOD) in an attempt to offset this. Among the several processes causing this stress are glycoxidation, non-enzymatic glycation, Aldose Reductase (AR) activation, and the activation of certain enzymes and inflammatory pathways. These factors reduce the effectiveness of the lens's antioxidant defences, hence increasing oxidative damage[7]. More precisely, increased AR activity is linked to lipid peroxidation and antioxidant depletion-e.g., ascorbate and GSH. Non-enzymatic glycation can interfere with the action of antioxidant enzymes; an increase in inducible nitric oxide synthase might generate possibly harmful chemicals such as nitrotyrosine[8]. A major factor in cataract development is oxidative stress, which is exacerbated by radiation (including UV rays), vitrectomy, and age. Oxidative stress on lenses lowers glutathione levels and causes the synthesis of protein-thiol mixed disulfides such as PSSG (Protein-S-S-Glutathione). Proteins cross-link during this process and lose their water-soluble properties. The final result of this process is lens opacification, or cataract formation, which aggregates highly molecular weight proteins[9].

The tea plant leaves, *Camellia sinensis* L. (*Theaceae*), provide a great supply of antioxidant compounds including gallocatechin, epigallocatechin, epicatechin, and catechin. Long connected with *Camellia sinensis* include antimicrobial, anti-inflammatory, antifungal, anti-asthmatic, and antidiabetic activity[10,11].

The prospective anti-cataract properties of the leaf of the *Camellia sinensis* plant were investigated in this work using an *in vitro* model of glucose-induced cataract in goat eye lenses. As part of the study effort aimed at cataract prevention and antioxidants, several elements of the lens—including its shape, total protein content, catalase activity, and particular antioxidant activity—were examined[11,12].

2. Experimental Materials and Procedures

2.1 Collection and Verification of Plant Material

From Bhavnagar city, near Talaja, *Camellia sinensis* L. leaves were collected. The plant's verification was conducted by RK University's Department of Botany, School of Science in Gujarat.

2.3 Preparation of Plant Extract

To make powder, the plant leaves are air-dried at room temperature. Later, the powder was 48-hour methanol macerated. After evaporation at 40°C dried them, the extracts were kept at 4°C. As earlier mentioned, the

phytochemical components glycosides, phytosterol compounds, and flavonoids were evaluated[13,14].

2.4 Chemicals and Reagents

We obtained glucose from the ASTRON lab (based in India). The suppliers of the Total Protein and Catalase kits are AUTOSPAN®. Every chemical used arrived in analytical grade.

2.5 Collection of Goat Eye and Lens Culture

Fresh goat eyeballs came from the slaughterhouse in Vadodara. The abattoir removed the eyeballs when the animals died and kept them between 0 and 4 degrees Celsius. The lenses were grown in synthetic aqueous humour following extracapsular removal. Combined for 72 hours was a pH 7.8 solution, including 140 mM NaCl, 5 mM KCl, 22 mM $MgCl_2$, 0.5 mM $NaHCO_3$, 20.5 mM NaH (PO_4), 0.4 mM $CaCl_2$, and 5.5 mM glucose. Penicillin at 32 mg/L % and streptomycin at 250 mg/L % were included in the culture media to prevent bacterial contamination[13].

2.6 Generating Glucose-induced Cataract Model

A 55 mM glucose experiment was used to try to cause cataracts. The lens was dehydrated and exposed to oxidative stress because it converted its abundant glucose into polyols, or sugar alcohols, via the sorbitol route. This gave rise to cataract genesis. The experiment included *Camellia sinensis* extract dilutions and Dimethyl Sulfoxide (DMSO)[13,14].

2.7 Experimental Design of Groups

The six groups were created using a total of thirty-six lenses as described in Table 1.

2.8 Analysing the Lens Opacity in Photographs

After incubating the lenses for 72 hours, their back surfaces were put out on graph paper to measure their opacity. The following criteria were used to assess the opacity level[13,15].

0: Means there is no opacity;
+: Means there is a slight amount of opacity;
++: Diffuse opacity is present:
++++: Thick opacity is present in great amounts:

Table 1. Design of experiment groups (n=6)

Group I: Normal control glucose level is 5.5 mM
Group II: The *in vitro* Disease control group is 55 mM glucose
Group III: 55 mM Glucose + 12 ng/ml enalpril
Group IV: 55 mM Glucose + 250 µg/ml extract of tea leaf
Group V: 55 mM Glucose + 500 µg/ml extract of tea leaf
Group VI: 55 mM Glucose + 1000 µg/ml extract of tea leaf

2.9 Creating the Lens Homogenate

10 % of the lenses were homogenised in a sodium phosphate buffer with a pH of 7.4 and a concentration of 0.1 M following incubation for 72 hours. After refrigeration and thirty-minute centrifugation under 10,000 g at -4 degrees Celsius, the homogenate was used to support the next studies, and the recovered supernatant was then kept at -20 degrees Celsius[16,17].

2.10 Biochemical Parameter

Among the biochemical criteria calculated for the supernatant were total protein and catalase activity. The Biuret method helped one ascertain the lens's overall protein content. UV spectrophotometry tests were used to evaluate catalase activity level.

2.11 Statistical Analysis

The GraphPad Prism 9 program was used for the statistical study, applying a one-way analysis of variance (ANOVA). For every group of six lenses, the findings show the mean ± SEM (standard error of the mean). Considered statistically significant were P values below 0.05.

3. Results

Given that oxidative stress is a component in cataract development, human ophthalmology has also come to understand the significance of antioxidants in cataract prevention. Oxidative stress affects proteins, lipids, and DNA; cataracts are well-documented to change all three of these. Three molecular events—oxidative stress, an active polyol route for glucose clearance, and a lack of enzymatic glycation of eye lens proteins—may help to initiate diabetic cataract. All of these modifications speed up the creation of Reactive Oxygen Species (ROS) and raise the levels of oxidative chemical changes of proteins, DNA, and lipids in the

lenses of diabetic patients[18,19]. Using an *in vitro* model of glucose-induced goat eye lens damage, we studied the antioxidative and anticataract characteristics of *Camellia sinensis* L. leaf.

3.1 Lens Opacities Evaluated using a Photograph

Images taken using both standard and experimental lenses were examined using graph paper after a 72-hour incubation period. Compared with normal group and treatment group lenses, Group II (toxic control) lenses, submerged in 55 mM glucose, showed complete opacity (Figure 1, Table 2). By the end of the 72 hours, the lens's opacity had changed from an outward to an inward direction. The clarity of the lenses seen directly corresponds to the leaf extract efficacy when the lenses were incubated with a methanol extract of *Camellia sinensis* leaves. Group IV, then Group V, and finally Group VI showed the best clarity. The usual group III – Enalapril at a dose of 12 ng/ml with glucose did not induce lens growth (Table 2 and Figure 1).

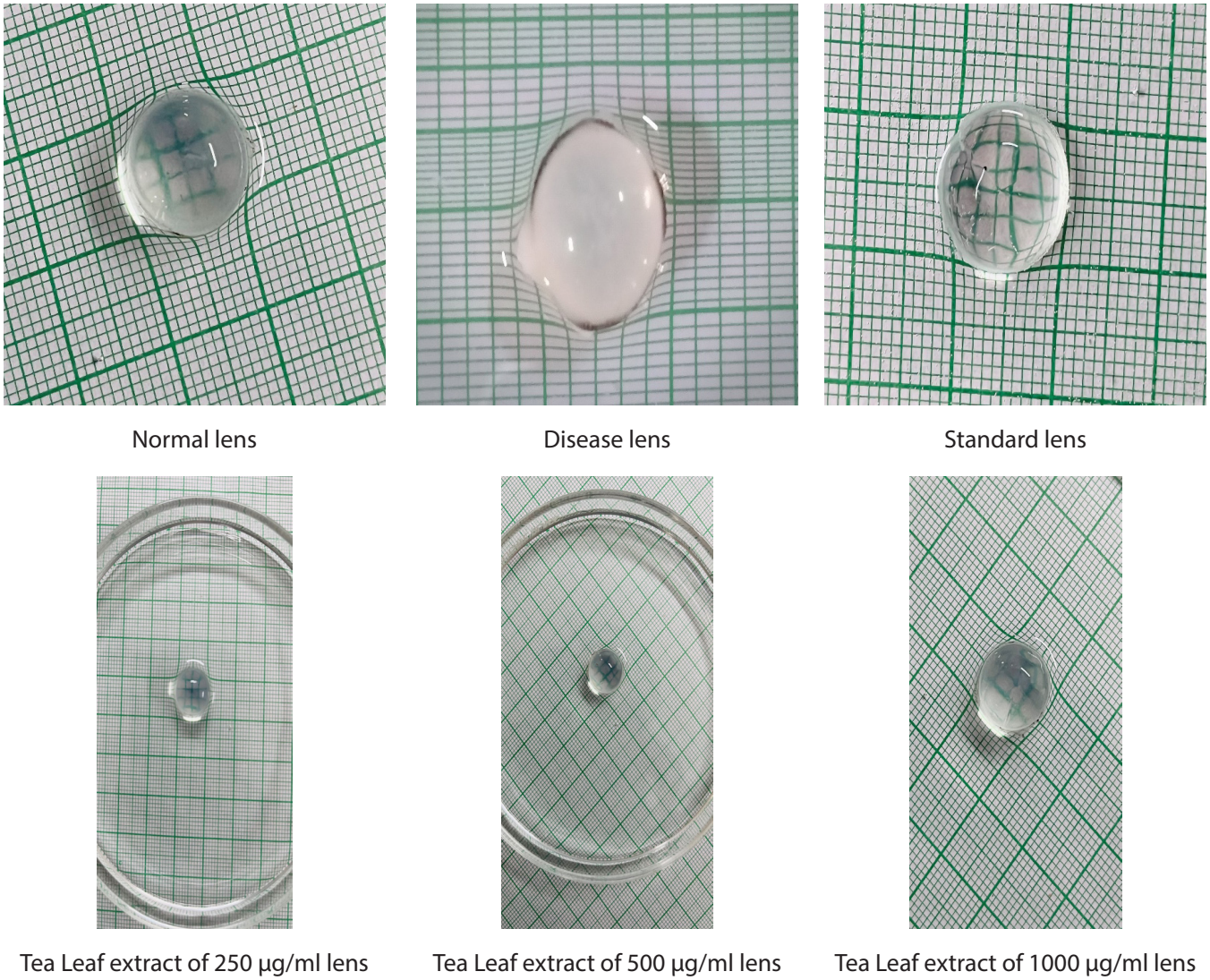


Figure 1. Incubation of images taken 72 hours following an extract from *Camellia sinensis* leaves has allowed evaluation of lens cloudiness. The solution from Group I has 5.5 mM glucose. Group II solely consists of 55 mM glucose. Along with 55 mM glucose, Group IV also consists of 250 µg/ml *Camellia sinensis* extract. Group V consists of 500 µg/ml *Camellia sinensis* extract with 55 mM glucose. Group VI calls for 1000 µg/ml *Camellia sinensis* extract along with 55 mM glucose. At last, Group II has 55 mM glucose plus 12 ng/ml Enalapril.

Table 2. 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 5.5 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 leaf extract of *Camellia sinensis*	+
5	Group V: 55 mM glucose plus 500 µg/ml leaf extract of *Camellia sinensis*	0
6	Group VI: 55 mM glucose plus 1000 µg/ml leaf extract of *Camellia sinensis*	0

3.2 Total Protein Level

The protein level increased in lenses from Groups I, III, which do not cause cataracts (Table 2 and Figure 2). Without the *Camellia sinensis* extract, Group II displayed low protein levels and thick opacity. When compared to the treated lenses, total protein levels in the sickness group were noted to be substantially higher (Fig. 2). Measuring Group II's recorded total protein levels, they came out to be (1.95 ± 0.17g/dl). Group IV, Group V, and Group VI—treated with varying doses of *Camellia sinensis* extract—3.13 ± 0.39* g/dl, 4.19 ± 0.28* g/dl, and 4.40 ± 0.37* g/dl, respectively, showed considerably greater total protein levels. These values increase above the group whose induced illness stemmed from glucose.

3.3 Catalase Activity

Table 2 and Figure 3 show that the Catalase activity increased in Group I lenses—lenses free of cataract formation. Without the sample extract, Group II showed thick opacity and a lower Catalase activity. Treatment on lenses using the extracts resulted in a far higher degree of catalase activity than in the disease group (Figure 3). Group II's recorded Catalase activity levels were found to be (1.30 ± 0.17#), while Group IV (1.83 ± 0.21*), Group V (2.27 ± 0.19*), and Group VI (2.65 ± 0.14 %). U/min/g.

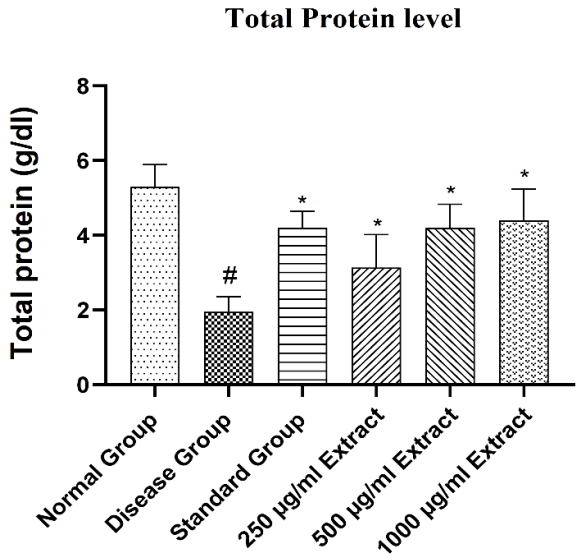


Figure 2. The overall protein level of Group II showed a clear decline from Group I. Whereas in those treated with Enalpril 12 ng/ml (Group –III), *Camellia sinensis* extract treatment (Group –IV, V, VI) was greatly raised as compared to (Group –II) total protein level. P= 0.05. Mean and SEM help to illustrate all values—n=6.

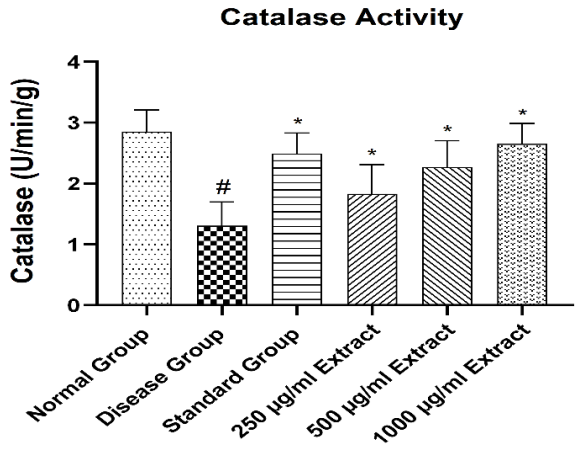


Figure 3. The catalase activity level in Group II showed a clear drop from Group I. Whereas that in treated with Enalpril 12 ng/ml (Group –III), *Camellia sinensis* extract treated (Group –IV, V, VI) was considerably greater than (Group –II) catalase activity level. P <0.05 is (Mean and SEM show all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

4. Discussion

Several studies have shown that hyperglycaemia is linked to a drop in lens clarity. Patients with hyperglycaemic issues run the risk of developing temporary oedema and lens opacification from the fast drop in serum glucose levels. Although the cataract surgical operation has advanced significantly, people with diabetes would not have the same degree of success as those without diabetes. Retinopathy, vitreous haemorrhage, iris neovascularisation, and vision

loss—either partial or total—have been reported 20. Among the negative effects that have been noted are these. The main cause of cataracts linked with ageing is oxidative stress resulting from the natural generation of free radicals in the environment of lens environment. Among the several kinds of free radicals, some are hydrogen peroxide, lipid hydroperoxides, and superoxide anion. Among the naturally occurring elements of young lenses are glutathione, vitamin C and E, carotenoids, and superoxide dismutase. These elements all help to explain the high antioxidant concentration in young lenses, which could help to avoid damage. It contains not just glutathione peroxidase and catalase but also additional antioxidant enzymes[21]. Loss of the antioxidant systems of the lens is one of the main risk factors for cataractogenesis, which is brought on by excessively high glucose levels in the blood and the formation of free radicals, including superoxide ion (O_2^-) and H_2O_2. High glucose levels are well-known to stimulate antioxidant enzymes, therefore indicating oxidative stress in the cell[22]. The lens culture used in the present investigation revealed a lens total protein, catalase activity level that was noticeably lower than that of a normal lens (Group I), with 5.5 mM of glucose. Among the groups treated with the extract, antioxidant activities increased; in Group III, the lens culture treated with enalapril and different concentrations of *Camellia sinensis* leaves extract exhibited noticeably greater levels of total protein and catalase activity. This helped to clarify the delay of the cataractogenesis process begun by high glucose concentration.

5. Conclusion

A leaf extract derived from *Camellia sinensis* has shown considerable antioxidant activity due to compounds including gallocatechin, epigallocatechin, epicatechin, and catechin, are exist, and anticataract activity in goat lenses separated and generated with diabetes cataract utilising glucose. Still under active research is the potential to prevent diabetic cataract utilising *Camellia sinensis* leaves. Moreover, increasing our knowledge in this field requires more study, including numerous *in vivo* animal models.

6. Author Contribution

In this study, Dilsar Gohil was responsible for conceptualizing the research idea, designing the study framework, and supervising the overall project. Ujjval Vaghela conducted the experimental work, collected the data, and contributed to the development of the methodology. Other authors performed the statistical analyses, interpreted the results, and prepared the figures and tables. All authors collaboratively drafted the initial version of the manuscript, while Dilsar Gohil provided critical revisions to improve its intellectual content. All authors contributed to the review and editing of the final manuscript, approved its submission, and agreed to be accountable for all aspects of the work.

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