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SHORT COMMUNICATION

Phytotherapeutic Investigation of *Leucas aspera* Leaf Extract for Preventing Glucose-induced Cataractogenesis in Goat Lenses

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Abstract

Background: Diabetes mellitus is vital factor in cataract development, due to generation of oxidative stress and free radical. Natural phytoconstituent are free radicals scavenger, retard the progression of cataract formation. Thus, utilization of natural plants as a source of antioxidants is highly promising for cataractogenesis prevention. **Aim:** To investigate the anti-oxidant parameter MDA content, catalase activity and total protein content in glucose induced cataractogenesis *in vitro* goat eye lenses and identifying the role of *Leucas aspera* leaves extract in prevention cataractogenesis. **Methods:** In this *in-vitro* model, collected lenses were categorized into 6 groups (n=6). Normal group (Glucose: 5.5 mM) as Group I while other 5 groups were given glucose (55 mM) treatment to create an experimental cataractogenesis. Group II: Disease group (cataract lenses). Group III exhibited Enalapril 13 ng/ml. Group IV lenses were exposed to 200 µg/ml extract of *L. aspera* leaves; Group V lenses were exposed with a 400 µg/ml preparation of *L. aspera* leaves. While Group VI lenses were exposed with a 600 µg/ml extract of *L. aspera* leaves. Prepared tissue lens homogenates were used to evaluate lipid peroxidation (MDA), catalase activity, and total proteins content. **Results:** *L. aspera* exhibited a considerable increase in antioxidant enzyme activity, total protein preservation, catalase levels, and Malondialdehyde (MDA) reduction. Furthermore, *L. aspera* leaf aqueous extracts attenuated the opacity development in the lenses. Conclusion: In this investigation, *L. aspera* showed anti-oxidant and anticataract properties *in vitro* glucose-induced goat eye lens model. **Major Findings:** The study found that *L. aspera* leaf extract significantly enhanced antioxidant enzyme activity, reduced MDA content contributing to reduced lens opacity, suggesting its promising role of anti-cataract and antioxidant properties.

Keywords: Anticataract Activity, Anti-oxidant, Catalase Activity, Goat Eye, *L. aspera*, Lens Opacity, Malondialdehyde Level

1. Introduction

A cataract is opacity of the transparent lens of the eye, which impairs vision. This condition is prevalent more in diabetic patient due to increase HBA1c levels[1]. Globally, cataracts are the primary cause of blindness. This condition is attributed to a range of variables such as Ultraviolet (UV) radiation, dietary insufficiency, infectious disorders, aging, exposure to chemicals and medications, and genetic defects[2] a leading cause of blindness worldwide, is a multifactorial eye disease. In developing countries the incidence of cataract among young generations is not uncommon due to malnutrition, excess exposure to ultraviolet radiation and so on. In developed countries, age-related cataract affecting the population over 65 years of age is a major concern. Oxidative stress was suggested to inflict damage to the lens and induce opacification, and a variety of antioxidant nutrients were tested for the prevention or delay of cataract development. Although promising results were obtained in animal studies of various antioxidants, epidemiological studies on human populations do not seem to support their protective effects unequivocally. It is unlikely that age-related

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cataract in man, similar to the ageing process itself, will be prevented or delayed by therapeutic drugs in the foreseeable future. At present, keeping a health-conscious life style (i.e., no smoking.

Multiple research studies reveals, over one-third of global populations suffering from blindness, where cataract is solely responsible[2,3]. Additionally, 55-60 million people suffer from moderate or severe vision impairment. Aging process and diabetic individuals have a fivefold increased likelihood of getting cataracts at an earlier age compared to those without diabetes. Potential risks encompass accelerated advancement of diabetic retinopathy, photic retinopathy, and the worsening of macular oedema. Current pharmaceutical market lacks any pharmaceutical intervention for the prevention or treatment of cataracts. Further intricate surgical interventions are only tool to address the chronic stage of diabetic individuals with cataracts, and who also suffer from slow healing process after surgery when compared to normal individuals. Diabetic patient and cataracts together creates greater challenges demanding lenses replacement, adverse effect associated with the materials used in some intraocular implants[4]. Despite of the remarkable advancements in synthetic chemistry, certain natural ingredients remain in pharmaceutical formulations due to their usage and easy availability[5] morphine, codeine and other compounds with beneficial effects. Pure compounds enabled accurate dosing to achieve serum levels within the pharmacological window and reproducible clinical effects. During the 20th and the 21st century synthetic compounds became the major source of drugs. In spite of the impressive results achieved within the art of synthetic chemistry, natural products or modified natural products still constitute almost half of drugs used for treatment of cancer and diseases like malaria, onchocerciasis and lymphatic filariasis caused by parasites. A turning point in the fight against the devastating burden of malaria was obtained in the 17th century by the discovery that bark from trees belonging to the genus Cinchona could be used for treatment with varying success. However isolation and use of the active principle, quinine, in 1820, afforded a breakthrough in the treatment. In the 20th century the synthetic drug chloroquine severely reduced the burden of malaria. However, resistance made this drug obsolete. Subsequently artemisinin isolated from traditional Chinese medicine turned out to be an efficient antimalarial drug overcoming the problem of chloroquine resistance for a while. The use of synthetic analogues such as chloroquine or semisynthetic drugs such as artemether or artesunate further improved the possibilities for healing malaria. Onchocerciasis (river blindness. Modern cataract surgery in diabetes people may cause vitreous haemorrhage, promote iris neovascularisation, cause diabetic retinopathy to proceed very quickly, and finally result in vision loss. Plant-based substances such as curcumin, rutin and luteolin have traditionally provided the majority of the active components in pharmaceuticals. When applied to drug development in days before high-throughput testing and the post-genomic age, it is well acknowledged that over 80% of drug compounds were made from plants or derived from a natural chemical[6].

Dronapushpi, scientifically known as *L. aspera* (Willd) Linn., belongs to the *Lamiaceae* family and is a plant species that is widely found in India, ranging from the Northern Himalayan region to Southern Sri Lanka of Asia. The plant has been used as a remedy for reducing fever and as a substance for killing insects. Leaves are beneficial for treating chronic Rheumatism, Psoriasis, and other persistent skin problems. Locally, bruised leaves are typically used for snake bites[7]. The plant has been historically used as a febrifuge and insecticidal agent. Scientifically, it has been shown to exhibit a range of pharmacological properties, including antifungal, antioxidant, antimicrobial, antibacterial, antinociceptive, cytotoxic action, protease and thrombolytic action, anti-ulcer, anti-inflammatory, hepatoprotective, anti-asthmatic, analgesic, immunomodulatory, anthelmintic, antidiabetic, CNS depressant, antipyretic, anti-hyperlipidaemic, anti-venom, anti-rheumatic, and anti-psoriatic[8-11]. Current study was designed to identify the anti-cataract potential of *L. aspera* leaves extract on glucose-induced diabetic cataracts in *in-vitro* goat eye lens.

2. Materials and Methods

2.1 Materials

Fresh leaves of *L. aspera* were obtained from land area in Palayamkottai village, Tirunelveli district, Tamil Nadu, India. The authentication of the plant was done

at Department of Biosciences, Saurashtra University, Rajkot. All chemicals, antibiotics and reagents of analytical grade were bought from Chemdyes corporation, India.

2.2 Methods

2.2.1 Preparation of Plant Extracts and Phytochemical Screening

Cold maceration techniques was used for extraction[12,13]. Collected leaves were properly washed and they were allowed to air dry at room temperature to avoid the enzymes from becoming denatured. Weighed 50 g plant powder was homogenized with ice-cold methanol in a beaker, kept aside with frequent shanking for duration of 72 hrs. Further, methanol extract was filtered using Whatman's filter paper No. 1, centrifuged at 6,000 rpm at 4° C for 15 minutes. The collected extract was stored at 4-5° C and employed for phytochemical screening[14].

2.2.2 Preparation of Lens Culture

With extracapsular cataract extraction technique, the goat eye lenses were collected from the slaughterhouse and immersed cold artificial aqueous humour comprising 140 mM NaCl, 2 mM $MgCl_2$, 5 mM KCl, 0.5 mM $NaHPO_4$, 0.5 mM $NaHCO_3$, 0.4 mM $CaCl_2$, and 5.5 mM glucose, were maintained at a temperature of 37° C and pH level of 7.8, for 72 hours. 250mg of each penicillin and streptomycin antibiotics were added for bacterial growth inhibition[15].

2.2.3 Experimental Design

The concentrations of *L. aspera* extract (200, 400, and 600 µg/ml) were selected to establish a dose-response relationship, a fundamental approach in pharmacological studies, and are within the range of concentrations used for similar plant extracts in other antioxidant assays. This range was deemed appropriate to observe potential concentration-dependent effects while minimizing the risk of non-specific cytotoxic effects. The collected Goat lenses were divided into 6 experimental groups (n=6) as follows:

Group I: Normal group (Aqueous humour+ Glucose 5.5 mM)

Group II: Disease group (Aqueous humour+ Glucose55 mM)

Group III: Standard group (Aqueous humour + Enalapril (13ng/ml) + Glucose 55 mM)

Group IV: LRE200 group (Aqueous humour + *L. aspera* (200 µg/ml) + Glucose 55 mM)

Group V: LRE400 group (Aqueous humour + *L. aspera* (400 µg/ml) + Glucose 55 mM)

Group VI: LRE600 group (Aqueous humour + *L. aspera* (600 µg/ml) + Glucose 55 mM)

2.2.4 Lens Homogenate Preparationand Photographic Assessment of Lens Opacity

The lenses were incubated for about 72 hours and opacity was analysed by placing the posterior surface of the lenses on the laminated graph paper. The degree of opacity was graded as follows[15].

(0) Means 0 Opacity - graph lines visible.

(+) Means 1 Opacity- graph lines mostly visible.

(++) Means 2 Opacity - graph lines partially visible.

(+++) Means 3 Opacity - graph lines not visible.

Lens homogenate was prepared by using Tri's buffer (pH 7.2), containing EDTA, centrifuged at 10,000 rpm at 4° C for 15-20 minutes in ice-cold condition. Isolated supernatant was used in estimation of biochemical parameters[16].

2.2.5 Biochemical Parameter

All biochemical parameters total protein content, malondialdehyde and catalase activity was estimated in supernatant by Biuret test, TBA test and Von Euler and Josephson protocol respectively[16,17].

2.2.6 Statistical Analysis

Results are presented as mean +/- SEM for each group (n=6). $P < 0.05$ was considered as statistically significant results. All data were analysed using Graph pad prism 9 software using one -way ANOVA (Analysis of variance).

3. Results and Discussion

The importance of antioxidants in preventing cataracts is well-recognized in human eye health, as oxidative stress is a key factor in cataract formation. Oxidative stress can damage proteins, lipids, and DNA in the eye, all of which contribute to cataract development. In diabetic cataracts specifically, three molecular

Table 1. Phytochemical analysis of aqueous extract of *L. aspera* leaves

S. No.	Phytochemical Analysed	Result
1	Carbohydrates	+
2	Alkaloids	+
3	Phenols	+
4	Saponins	-
5	Glycosides	+
6	Steroids	+
7	Tannins	-
8	Flavonoids	+
9	Terpenoids	+
10	Proteins	-

mechanisms are thought to be involved: Oxidative stress, an overactive polyol pathway that removes glucose, and insufficient enzymatic glycation of lens proteins[18]. These processes increase the oxidative stress to proteins, DNA, and lipids in the eye lens of diabetic patients and accelerate the production of Reactive Oxygen Species (ROS). Various phytoconstituents such as Apigenin, Luteolin, Quercetin, Leucasperosides A, Leucasperosides B and Leucasperosides C presence (Table 1) shows the potential of plant in prevention in cataract formation[19]. To evaluate the antioxidative and anticatalytic potential of *L. aspera* leaf, we conducted an *in vitro* study using glucose-induced damage in a goat eye lens model

Table 2. Photographic evaluation of lens opacities - degree of opacity (n=6)

S. No.	Groups	Treatment	Degree of Opacity
1	Group 1	Normal group (glucose 5.5 mM)	0
2	Group 2	Disease group (glucose 55 mM)	++++
3	Group 3	Standard group (13ng/ml)	0
4	Group 4	LRE 200 (200 ug/ml))	+
5	Group 5	LRE 400 (400 ug/ml))	+
6	Group 6	LRE 600 (600 ug/ml))	0

Post 72 hours of incubation, both standard and test group lenses were analyzed using graph paper for clarity assessment. Lenses in Group II (Disease control), exposed to 55 mM glucose, and displayed full opacity, unlike lenses in the normal and treatment groups (Figure 1, Table 2). Over the incubation period, opacity progression moved from the lens edges inward. Among treated groups, Group IV showed the highest lens clarity, followed by Groups V and VI, when lenses were treated with methanolic extract from *L. aspera* leaves. The clarity observed was directly proportional to the efficacy of the leaf extract. Group III is treated with 12 ng/ml Enalapril in glucose, did not show lens opacity (Table 2 and Figure 1).

Group I: Aqueous humour containing 5.5 mM glucose. Group II: Aqueous humour of 55 mM glucose. Group IV: Aqueous humour contains 200 µg/ml extract of *L. aspera* in addition to 55 mM glucose. Group V: Aqueous humour includes 400 µg/ml extract of *L. aspera* with 55 mM glucose. Group VI: Aqueous humour involves 600 µg/ml extract of *L. aspera* combined with 55 mM glucose. Lastly, Group III: Aqueous humour contains 55 mM glucose along with 13 ng/ml Enalapril drug.

In Group II, MDA levels measured 7.690 ± 1.67 µmol/ml. However, lenses treated with *L. aspera* methanolic extract showed a marked reduction in MDA levels across different concentrations. At 200 µg/ml, the MDA level decreased to 1.649 ± 0.145 µmol/ml; at 400 µg/ml, it further dropped to 5.780 ± 2.398 µmol/ml; and at 600 µg/ml, it reached 2.436 ± 2.147 µmol/ml. These reductions were significant when compared to Group II (Figure 2) In Groups I and III, which did not develop cataracts, protein levels were shown to increase (Figure 3). Conversely, Group II, which lacked treatment with *L. aspera* extract, exhibited dense opacity and lower protein levels (Figure 3). When comparing the disease group to the treated groups, total protein levels were significantly higher in the treated lenses (Figure 3). Specifically, total protein in Group II measured 1.20 ± 0.49 g/dl, while Groups IV, V, and VI-treated with various concentrations of *L. aspera* extract-demonstrated substantially higher protein levels: 3.50 ± 0.49 g/dl, 5.32 ± 0.33 g/dl, and 6.67 ± 0.36 g/dl, respectively. These results indicate a significant increase in protein levels compared to the glucose-induced disease group. In Group I lenses

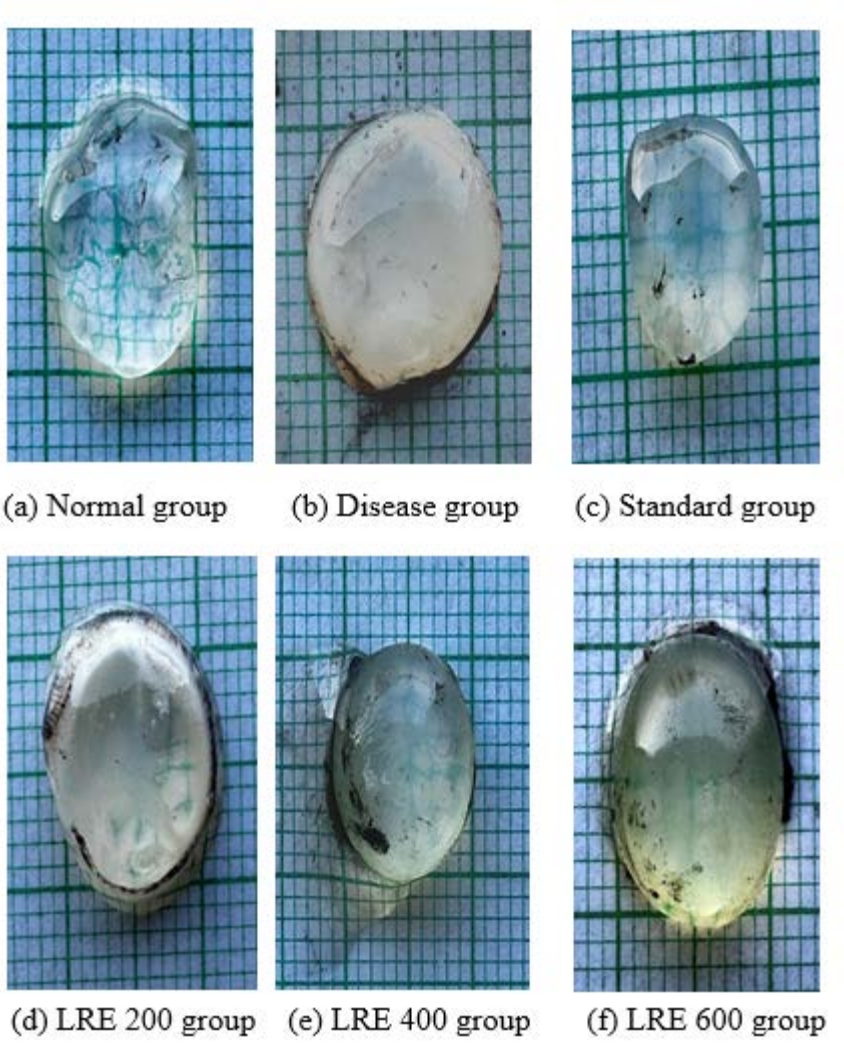


Figure 1. Observation of lens opacity through photographically, following 72-hour incubation duration with *L. aspera* leaves extract.

(those without cataract formation), catalase activity was observed to increase (Figure 3). In contrast, Group II, which did not receive the sample extract, showed reduced catalase activity and exhibited dense opacity.

Group-II showed significant increase Lipid peroxidation (MDA) when compared to (Group-I). Whereas in the group treated with Enalapril 12 ng/ml (Group-III), and *L. aspera* extract treated (Group-IV, V, VI) was significant decreased as compared to (Group-II) MDA level. (P <0.05) Mean and SEM are used to represent all values (n=6).

Group-II showed significant decrease in total protein content when compared to (Group-I). Whereas that in treated with Enalapril 12 ng/ml (Group –III), and *Leucas aspera*extract treated (Group-IV, V, VI) was significant increased as compared to (Group-II) Total protein content. (P <0.05) Mean and SEM are used to represent all values (n=6).

Treatment with the extracts significantly boosted catalase activity in the lenses compared to the disease group (Figure 4). Specifically, Group II had catalase activity levels of 0.762 ± 0.19 U/min/g, while treated groups showed notably higher levels: Group IV at 1.46 ± 0.26 U/min/g, Group V at 1.86 ± 0.14 U/min/g, and Group VI at 2.17 ± 0.20 U/min/g.

Group-II showed significant decrease in catalase activity when compared to (Group-I). Whereas in the group treated with Enalpril 12 ng/ml (Group-III), and *L. aspera* extract treated (Group-IV, V, VI) was significant increased as compared to (Group-II) total

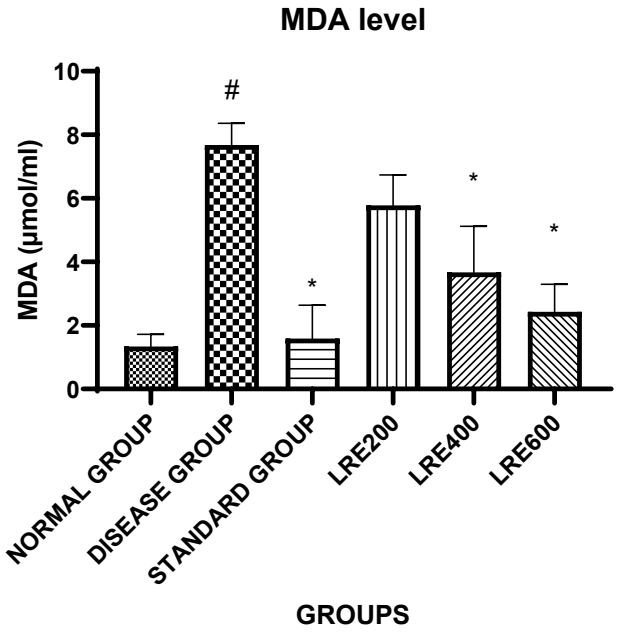


Figure 2. MDA assessment.

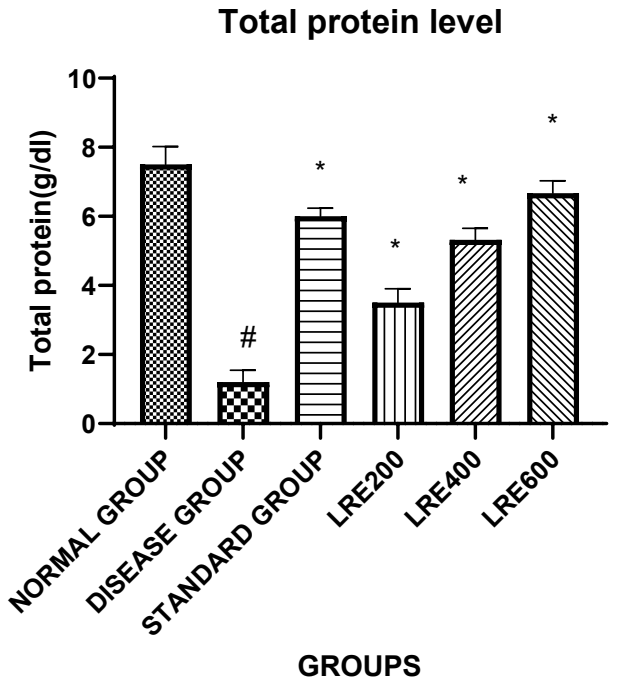


Figure 3. Total protein assessment.

protein content. (P <0.05) Mean and SEM are used to represent all values (n=6).

Research study suggests there is a link between hyperglycaemia and decreased lens clarity. In

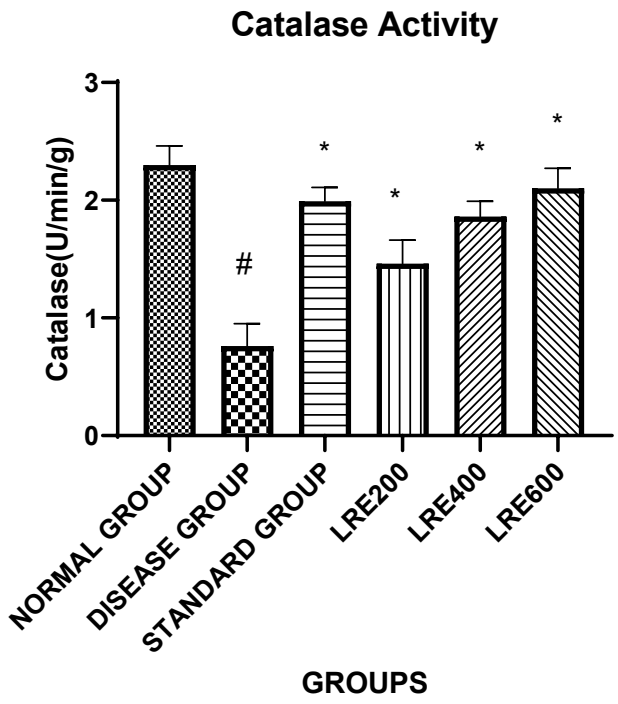


Figure 4. Catalase activity.

hyperglycaemic patients, rapid drops in serum glucose levels can lead to temporary lens swelling and opacification[20]. While advancements in cataract surgery have improved outcomes, diabetic patients often face complications, such as retinopathy, iris neovascularization, and even partial or total vision loss. Age-related cataracts are primarily caused by oxidative stress from free radicals like hydrogen peroxide, hydroxyl radicals, superoxide anion, and lipid hydroperoxides in the lens environment. Young lenses are naturally rich in antioxidants such as glutathione, vitamins like C and E and enzymes like SOD (Superoxide Dismutase), catalase, and Glutathione peroxidase (GH), which protect against damage [21]. However, as antioxidant defences decline, cataract formation risk rises, especially with high blood glucose, which promotes free radical generation, such as superoxide ions (O_2^-) and H_2O_2. High glucose also induces antioxidant enzyme activity, signaling oxidative stress within cells[22]. In this study, lenses cultured with high glucose levels showed elevated MDA levels and reduced catalase activity compared to normal lenses (Group I) exposed to 5.5 mM glucose. Lenses treated with enalapril and various concentrations of *L. aspera* leaves extract exhibited

higher total protein, catalase activity, and MDA levels than those in Group II. Additionally, antioxidant activity increased significantly in the extract-treated groups, helping to delay cataract formation induced by elevated glucose levels.

4. Conclusion

The leaf extract from *L. aspera* has shown notable antioxidant and anti-cataract effects in isolated goat lenses induced with diabetic cataracts *via* glucose exposure. Additional studies, on various *in vivo* animal models, will help us to further deepen our understanding of this plant potential.

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6. Author Contribution

Conceptualization and methodology: G. Santhana Kumar; Data collection, writing and experimentation: Kushal Solanki; Extraction: Parth B. Narigara. Investigation: Harshil Majethiya; Editing and draft preparation Meghna Seta; Resources and supervision: Lalji H. Baldaniya. All authors have read and agreed to the published version of the manuscript.

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