Anticataract effects of *Pergularia daemia* leaf on *in-vitro* glucose induced goat eye lens model

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Received: 29 June 2023
Revised: 6 August 2023
Accepted: 14 August 2023
Published: 22 November 2023

Egyptian Pharmaceutical Journal 2024, 23:103–109

Background
The primary factors contributing to the development of cataracts in diabetes mellitus are the generation of free radicals and oxidative stress. It is widely recognized that the progression of cataracts can be attenuated by the presence of antioxidants. The prospect of utilizing natural plants as a source of antioxidants is highly promising.
Objectives
In order to determine the antioxidant activity of MDA level, Catalase activity, and Total protein level as well as to evaluate the morphological changes in goat eye lens, we investigated the anticataract effects of *Pergularia daemia* leaf on an *in vitro* glucose-induced goat eye lens model.
Material and methods
Six groups of 36 each were created out of lenses. 'Normal Control' (5.5 mM) constituted Group I. The remaining five groups used glucose (55 mM) to create an experimental diabetic cataract. Group II: ' Disease Control' (experimental, untreated diabetic cataract lenses). Enalpril 12 ng/ml and 55 mM glucose make up Group III. Lenses treated with a 250 μg/ml extract of *Pergularia daemia* leaves were placed in group IV. Lenses treated with a 500 μg/ml preparation of *Pergularia daemia* leaves, group V. Lenses treated with a 1000 μg/ml extract of *Pergularia daemia* leaves are in Group VI. Malondialdehyde, a marker of lipid peroxidation, catalase activity, and total proteins were among the biochemical factors found in lens homogenates that were examined. In each group, the morphology of the lenses was compared.
Results
The antioxidant enzyme activity, preservation of total proteins, catalase levels, and reduction of malondialdehyde (MDA) were significantly increased in *Pergularia daemia*. Additionally, the methanolic extracts of *Pergularia daemia* leaves effectively delayed the development of opacity in the lenses while maintaining their shape.
Conclusion
The *in-vitro* glucose-induced goat eye lens model demonstrated anti-oxidant and anticataract capabilities as well as the preservation of lens shape in an anticataract study.

Keywords:
anticataract activity, anti-oxidant, catalase activity, malondialdehyde level, opacity

Egypt Pharmaceut J 23:103–109

1687-4315

Introduction

A cataract is a visual impairment that develops when the transparency of the lens is disturbed. It is one of the key health issues that has received some attention globally and is one of the leading causes of blindness. In underdeveloped nations, cataracts are a significant factor in blindness [1].

According to estimates, 75% of blindness worldwide might have been prevented due to the prevalence of treatable eye disorders or causes of blindness, such as cataract, trachoma, onchocerciasis, and other eye abnormalities in infants. There are 285 million visually impaired persons in the world, including 246 with low vision and 39 million blind [2]. Nearly half of all blindness worldwide is caused by cataracts [3,4].

The development of diabetic cataract is linked to a process called the polyol pathway. The sorbitol-aldose reductase route is another name for the polyol pathway. By way of the polyol pathway, aldose reductase catalyses the conversion of glucose to sorbitol. Numerous aldehydes and carbonyls can be reduced by the NADPH-dependent oxidoreductase enzyme known as aldose reductase. Because sorbitol cannot permeate cell membranes, it causes osmotic stress on cells by luring water into the tissues that depend on insulin [5]. Increases in intracellular and extracellular

sorbitol levels, as well as levels of reactive oxygen species, are brought on by excessive activation of the polyol pathway, although nitric oxide and glutathione levels are lowered. These abnormalities cause cellular damage and the production of opacity in diabetic patients. Oxidative stress is linked to increased production of oxidizing species are produced more often or antioxidant defense molecules like glutathione are significantly less effective [6]. However, more extreme oxidative stress results in necrosis, While milder oxidation can occasionally stimulate apoptosis. More severe oxidative stress can also cause cell death. By way of cross-linking, aggregation, and precipitation, oxidative stress directly modifies the inner lens proteins. Toxic aldehydes produced by oxidative damage to the sensitive retina and peroxidation of the lens epithelium may help cause the eventual opacity-causing damage to lens proteins Catalase (CAT), total protein, superoxide dismutase (SOD), glutathione peroxidase (GPx), and reduced glutathione (GSH), which are beneficial antioxidant enzymes, become less active as a result of cataract [7]. A high glucose level is one of the primary factors driving the onset of diabetic cataract. Glycation of sugars and reacting with amino groups to generate adducts interfere with proteins' ability to perform biologically [8]. As a result, structural changes are made to enzymes, which ultimately lead to enzyme deactivation. When under osmotic stress or hyperglycemia, the route becomes more active. When significant changes to the lens' opacity were made in experimental models of hyperglycemia, it was discovered that this condition is linked to persistent issues with diabetes [9].

Natural antioxidants are renowned for their ability to shield cells and living things against oxidative stress, which is believed to play a part in ageing and degenerative diseases. The phenolic compounds—phenolic acids and flavonoids—carotenoids, tocopherol, and ascorbic acid are the main antioxidants found in food, particularly vegetables [10,11].

The Asclepiadaceae family includes the most well-known species, *Pergularia daemia* (Forsskal) Chiov, whose leaves are a rich source of phytochemicals such quercetin, kaempferol, and formononetin phytosterols and cardiac glycosides. The potential for antioxidant, anti-inflammatory, antibacterial, and antidiabetic activities of *Pergularia daemia* formulations has been established [12,13].

The present study examined the potential anticataract properties of the *Pergularia daemia* leaf using an in-vitro model of glucose-induced cataract in goat eye lenses. Additionally, the study explored the antioxidant and cataract-preventive abilities & primarily focused on evaluating the lens's shape, total protein content, MDA level, catalase activity, and specific antioxidant activity.

Experimental materials and procedures

Collection and verification of plant material

From Rajkot city, near Aji-dam Park, *Pergularia daemia* (Forsskal) Chiov. leaves were collected. authentication of the plant was done at the Department of Botany School of Science at RK University in Rajkot, Gujarat.

Preparation of plant extract

In order to make powder, the plant leaves were air dried at room temperature. Afterward, 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 [14,15].

Materials

Methanol was bought from the RANKEM lab, and Glucose was bought from the ASTRON lab (in India). The Total protein, MDA, Catalase kits purchase from AUTOSPAN®. The chemicals utilized were all of the analytical grade.

Lens culture

Fresh goat eyeballs were brought from Sadar bazar, Slaughter house, Rajkot. Then, the eyeballs at a temperature of 0 to 4 degrees celsius from the abattoir as soon as the animals were killed. Extracapsular extraction was used to remove the lenses, and after that, they were cultured in artificial aqueous humour. NaCl 140 mM, KCl 5 mM, MgCl 22 mM, NaHCO3 0.5 mM, NaH (PO4) 20.5 mM, CaCl2 0.4 mM, and Glucose 5.5 mM were added to a solution with a pH of 7.8 and left at room temperature for 72 h. In order to avoid bacterial contamination, streptomycin 250 mg% and penicillin 32 mg% were added to the culture media [16,17].

Generating glucose-induced cataract model

Cataracts were generated using a 55 mM glucose concentration. Due to the high levels of glucose present in the lens, which were broken down via the sorbitol route, polyols (sugar alcohols) accumulated, the lens became overhydrated, and oxidative stress

developed. Cataractogenesis as a result was the result. with order to conduct the experiment, the extract was diluted with DMSO [16,17].

Groups design

A total of 36 lenses were used to produce the categories shown below (n=6):

Group I: Normal Group [5.5 mM glucose]
Group II: Disease Group [55 mM glucose].
Group III: Enalpril 12 ng/ml + glucose 55 mM
Group IV: Glucose 55 mM + 250 µg/ml of *Pergularia daemia* extract
Group V: 55 mM glucose + 500 µg/ml of *Pergularia daemia* extract
Group VI: 55 mM glucose + 1000 µg/ml of *Pergularia daemia* extract

Analyzing the lens opacity in photographs

The posterior surface of the lenses was laid out on a sheet of graph paper for 72 hours of incubation in order to evaluate their opacity. The level of opacity was evaluated using the following criteria [18].

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:

Preparation of lens homogenate

After a 72-hour incubation period, lenses from each group were extracted, and 10% of each lens was homogenized in a 0.1 M sodium phosphate buffer with a pH of 7.4. The resulting homogenate was then subjected to centrifugation at −4°C and 10,000 g for 30 min using a refrigerated centrifuge. The supernatant was collected and stored at −20°C for subsequent analysis [19].

Biochemical parameter

Malondialdehyde (MDA), total proteins, and catalase activity were among the biochemical parameters that were estimated for the supernatant. The Biuret method was used to estimate the amount of total protein in the lens, and the TBA test, von Euler and Josephson method, and 2-thiobarbituric acid test were used to measure the amount of MDA and catalase activity.

Statistical analysis

The statistical analysis was conducted using Graph-Pad Prism 9 software, employing a one-way analysis of variance (ANOVA). The results are presented as the mean±SEM (standard error of the mean) for each group of six lenses. P values less than 0.05 were considered statistically significant.

Results

The significance of antioxidants in cataract prevention has thus also been acknowledged in human ophthalmology, as the role of oxidative stress in cataract development has been recognized. Proteins, lipids, and DNA are all impacted by oxidative stress, and cataracts are known to modify all three of these. Oxidative stress, an active polyol route for the removal of glucose, and a deficiency in enzymatic glycation of eye lens proteins are three molecular mechanisms that may play a role in the onset of diabetic cataract. All of these alterations cause a rise in the amounts of oxidative chemical change of proteins, DNA, and lipids in diabetic patients' lenses, as well as a sped-up creation of reactive oxygen species (ROS) [20]. In order to test the antioxidative and anticatalytic effects of *Pergularia daemia* leaf, we used an *in vitro* model of glucose-induced goat eye lens damage.

Assessment of lens opacities by photograph

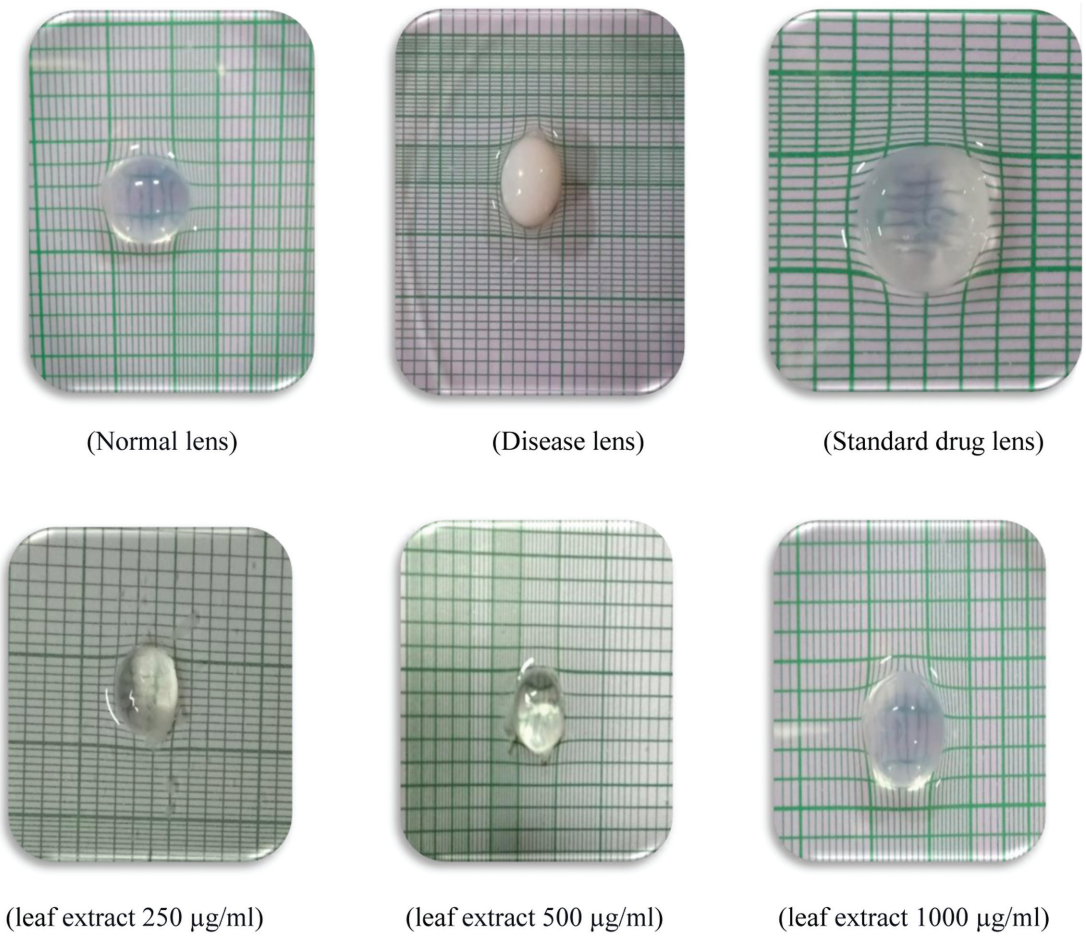
Following a 72-hour incubation period, graph paper was used to analyses the images taken with standard and experimental lenses. Comparing Group II (Disease control) lenses to Normal group and treatment group lenses, complete opacity was seen in Group II (toxic control) lenses placed in 55 mM glucose (Fig. 1, Table 1). By the end of the 72-hour period, the opacity in the lenses had moved from an outward to an inward direction. Best clarity was seen in Group IV, then Group V, and then Group VI when the lenses were incubated with a methanolic extract of *Pergularia daemia* leaves The clarity of the lenses viewed directly relates to the leaf extract efficacy. The usual group III – Enalapril at a concentration of 12 ng/ml with glucose did not cause the development of lenses (Table 1 and Fig. 1).

The impact of *Pergularia daemia* extract on indicators of oxidative stress

Malondialdehyde level (MDA)

One of the main factors causing cataractogenesis is oxidative stress. Plant extracts' ability to activate antioxidant defences helps to delay or even stop cataract development. Lipid peroxidation is the phenomenon where free radicals deprive lipids in cell membranes of their electrons. This process causes a decrease in membrane fluidity, an augmentation in membrane permeability, a deterioration in physiological function, and poses a serious threat to the cell's survival [Fig.2]. The substantial rise in glucose-induced cataractogenesis

Figure 1



Assessment of lens cloudiness through photography following a 72-hour incubation period with an extract derived from *Pergularia daemia* leaves. (Group I) comprises a solution containing 5.5 mM glucose. (Group II) consists solely of 55 mM glucose. (Group IV) contains 250 µg/ml extract *of Pergularia daemia* in addition to 55 mM glucose. (Group V) includes 500 µg/ml extract of *Pergularia daemia* with 55 mM glucose. (Group VI) involves 1000 µg/ml extract of *Pergularia daemia* combined with 55 mM glucose. Lastly, (Group II) contains 55 mM glucose along with 12 ng/ml Enalapril.

Table 1 Photographic evaluation of lens opacities – Degree of opacity (*n*=6)

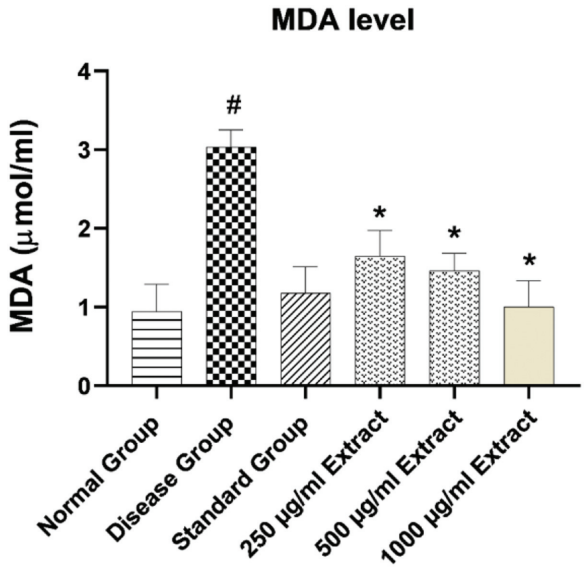
Sr. No.	Treatment with *Pergularia daemia* leaf extract	Degree of opacity
1	Group I – Normal lens – Glucose 5.5 mM	0
2	Group II – Disease lens – Glucose 55 mM	++++
3	Group III – Standard drug lens – Enalpril 12 ng/ml	0
4	Group IV – Glucose 55 mM + *Pergularia daemia* leaf extract 250 µg/ml	+
5	Group V – Glucose 55 mM + *Pergularia daemia* leaf extract 500 µg/ml	+
6	Group VI – Glucose 55 mM + *Pergularia daemia* leaf extract 1000 µg/ml	0

in Group II (3.039 0.095# µmol/ml) was observed. The lenses that were exposed to the methanolic extract of Pergularia daemia at a concentration of 250 µg/ml exhibited a significant alteration in their MDA levels when compared to the other lenses. (1.649 ±0.145* µmol/ml), 500 µg/ml (1.464±0.098* µmol/ml), 1000 µg/ml (1.006±0.147* µmol/ml) are to be decreased significantly as compare to disease group-II.

Total protein level

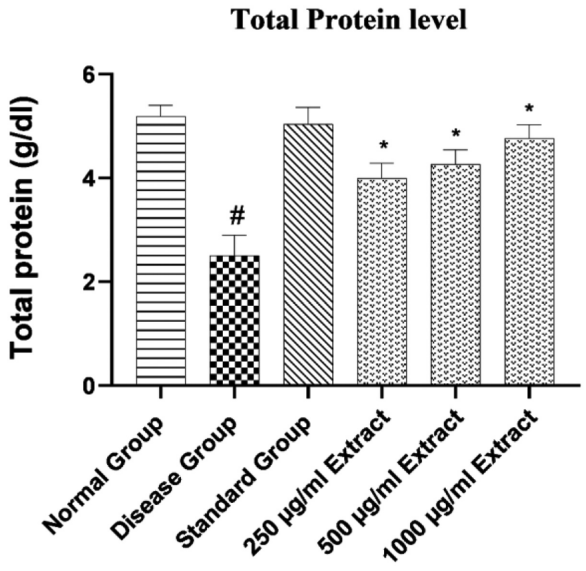
In lenses belonging to Groups I and III, which do not develop cataracts, it was shown that the protein level increased (Table 2). Group II, which did not contain the *Pergularia daemia* extract, displayed thick opacity and reduced protein levels (Fig. 1). Comparing the disease group to the treated lenses, it was found that the levels of total protein were significantly higher (Fig. 3). In Group II, the observed total protein levels were measured to be (2.50±0.39# g/dl). However, in Group IV, Group V, and Group VI, which were treated with various concentrations of *Pergularia daemia* extract, the total protein levels were significantly higher: (3.99 ±0.29* g/dl), (4.27±0.27* g/dl), and (4.77±0.25* g/dl), respectively. These values indicate a notable increase

Figure 2



Lipid peroxidation measured in term of malondialdehyde (MDA) level (Group – II) showed a significant increased as compared to (Group –I). whereas that in treated with Enalpril 12 ng/ml (Group –III), and *Pergularia daemia* 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).

Figure 3



Total protein level (Group – II) showed a significant decreased as compared to (Group –I). whereas that in treated with Enalpril 12 ng/ml (Group –III), and *Pergularia daemia* extract treated (Group –IV, V, VI) was significant increased as compared to (Group –II) total protein level. (P <0.05) Mean and SEM are used to represent all values (n=6).

Table 2 Using an isolated goat lens model, the effect of *Pergularia daemia* leaves on oxidative stress markers is demonstrated

Sr.no.	Parameter analyzed	Normal lens	Disease lens	Standard drug lens	leaf extract 250 µg/ml	leaf extract 500 µg/ml	leaf extract 1000 µg/ml
1	MDA level	0.94±0.15	3.03±0.095#	1.18±0.14	1.64±0.145*	1.46±0.098*	1.006±0.14*
2	Total Protein level	5.19±0.21	2.50±0.39#	5.04±0.32	3.99±0.29*	4.27±0.27*	4.77±0.25*
3	Catalase activity level	2.08±0.12	0.82±0.17#	1.99±0.18	1.61±0.16*	1.82±0.13*	1.87±0.12*

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

compared to the group that had induced disease with glucose.

Catalase activity

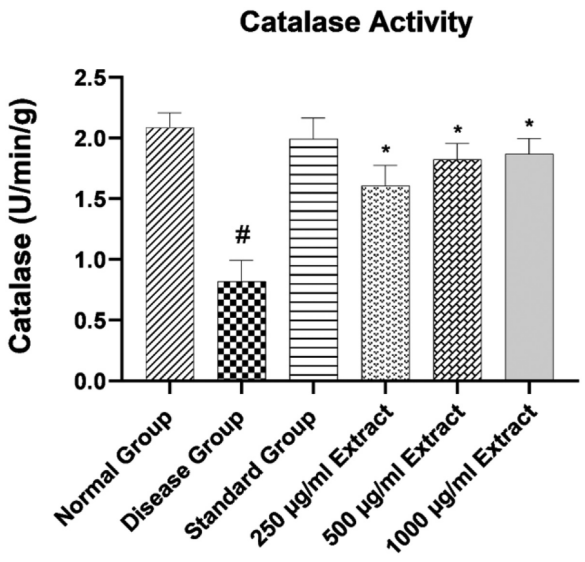
In Group I lenses (lenses without cataract development), the Catalase activity was seen to increase (Table 2). The Catalase activity was lowered and thick opacity was visible in Group II, which was devoid of the sample extract. When compared to the illness group, treatment with the extracts on lenses resulted in a significantly higher degree of catalase activity (Fig. 4). Catalase activity levels observed in Group II was found to be (0.822±0.17$^{\#}$) (U/min/g), while in treatment of Group IV (1.61±0.16*), Group V (1.82±0.13*), Group VI (1.87±0.12*) (U/min/g).

Discussion

According to several studies, hyperglycemia and the deterioration of lens clarity are related. When a patient has hyperglycemic issues, their serum glucose level drops quickly, which could cause temporary swelling and lens opacification. The success of cataract surgery has seen numerous improvements, but people with diabetes may not experience the same results. Some of the observed side effects include the occurrence of retinopathy, vitreous hemorrhage, iris neovascularization, and a decrease or complete loss of vision [21].

Age-related cataracts are the outcome of oxidative stress, which is brought on by the creation of free radicals in the lens environment. In addition to hydrogen peroxide and hydroxyl radicals, these free radicals also comprise superoxide anion and lipid hydro peroxides. The juvenile lens naturally has a lot of antioxidants, including glutathione, vitamin C and E, carotenoids, and superoxide dismutase, which may help to avoid damage. It also contains catalase, glutathione peroxidase, and other antioxidant enzymes [22]. The loss of the lens' antioxidant systems is one of the major risk factors for cataractogenesis, which is

Figure 4



Catalase activity level in (Group – II) showed a significant decreased as compared to (Group –I). whereas that in treated with Enalpril 12 ng/ml (Group –III), and *Pergularia daemia* extract treated (Group –IV, V, VI) was significant increased as compared to (Group –II) catalase activity level. ($P < 0.05$) Mean and SEM are used to represent all values ($n=6$).

triggered by an excessive amount of glucose in the blood and the production of free radicals such superoxide ion (O_{2-}) and H_2O_2. Antioxidant enzymes are known to be stimulated by high glucose levels, a sign that the cell is under oxidative stress [23].

The lens culture used in the current investigation had a lens MDA level that was substantially higher and catalase activity that was noticeably reduced when compared to a normal lens (Group I) with 5.5 mM of glucose. The lens culture treated with enalapril and different concentrations of *Pergularia daemia* leaves extract displayed noticeably higher levels of total protein, catalase activity, and MDA than in the lens culture of Group B, there was a noticeable rise in antioxidant activities among the groups treated with the extract. This contributed to the postponement of the cataractogenesis process triggered by high levels of glucose concentration.

Conclusion

A leaf extract derived from *Pergularia daemia* has demonstrated significant antioxidant and anticataract properties in goat lenses that were isolated and induced with diabetes cataract through the use of glucose. The investigation into the potential use of *Pergularia daemia* leaves for preventing diabetic cataract remains an active area of research. Further studies involving various *in vivo* animal models are also necessary to expand our understanding in this field.

Limitations

The antioxidant efficacy of *Pergularia daemia* leaf extract needs to be evaluated using different cataract models. To determine the potential future directions for in vivo studies, it is also necessary to conduct potency experiments on *Pergularia daemia* leaf extracts.

Acknowledgements

Financial support and sponsorship

Nil.

Conflicts of interest

The authors state that they have no conflicts of interest.

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