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Research Article

EVALUATION OF ANTICATARACT ACTIVITY OF *SCAEVOLA TACCADA* ON GLUCOSE-INDUCED CATARACT IN GOAT LENS: AN *EX VIVO* APPROACH

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ABSTRACT

Objectives: To evaluate of anti-cataract potential of *Scaevola taccada* in *ex vivo* Goat eye models.

Methods: Goat eye lenses separated into five groups were incubated in synthetic aqueous humor culture, added by streptomycin of 0.1 mg/mL was at 37°C for 72 h. Group I use as the normal control. In Group II (disease control), the lenses were added by 1 M glucose. The lenses in Group III (standard control) were incubated in an aqueous humor medium with 1 M glucose added by 500 µg/mL of Gallic acid. The lenses in Group IV (Test Group) were incubated in aqueous humor medium with 1 M glucose added by 500 µg/mL of extract of *S. taccada*. MDL levels and catalase activity were measured by using ultraviolet Spectroscopy. Statistical analysis of evaluation parameters was conducted using analysis of variance and *post hoc* Tuckey test.

Results: As a part of the study, in malondialdehyde (MDA) level estimation, our test drug has a low MDA level as compared to the disease control. Hence, it may be acting as an anti-cataract. In total protein estimation, the test drug has a higher amount of total protein in comparison with others. By analyzing catalase activity, Catalase activity data revealed that the test drug is having high catalase activity as compared to the standard control group. Hence, we conclude that it works as an anti-cataract.

Conclusion: The study carried out clearly revealed that *S. taccada* (Bhadrax) has the potential for anti-cataract activity. This hypothesis was supported by finding such total protein level, MDA level, and catalase activity.

Keywords: *Scaevola taccada*, Oxidative stress, Gallic acid, Anti-cataract activity.

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INTRODUCTION

Cataract is a common eye condition that affects millions of people worldwide [1]. It is a progressive clouding of the eye's natural lens, which leads to blurred vision and reduced visual acuity. Cataracts can develop in one or both eyes and can occur in people of all ages, although they are most commonly found in older individuals [2]. The human eye is composed of several structures that work together to form an image. The cornea, a clear domeshaped structure at the front of the eye, is responsible for bending and focusing incoming light [3]. The lens, located behind the iris and pupil, further refracts and focuses light onto the retina, a layer of light-sensitive cells at the back of the eye [3]. The retina then converts the incoming light into electrical signals that are sent to the brain, where they are interpreted as visual images [4]. A healthy lens is clear and transparent, allowing light to pass through it easily. However, over time, the lens can become cloudy and opaque, leading to the development of cataracts [5]. Cataracts can also form as a result of injury to the eye, certain medications, or medical conditions such as diabetes [6].

In treatment, typically necessitates surgical intervention involving the removal of the affected lens and replacement with an artificial one [7]. Despite its generally high success rate, cataract surgery does pose potential risks such as infection, bleeding, and anesthesia-related complications [7]. Various types of cataracts exist, including age-related, congenital, and secondary cataracts arising from other medical conditions or medications. Age, family history, smoking, and certain medical conditions like diabetes are identified as risk factors for cataract development [8]. Prevention strategies involve quitting smoking, managing medical conditions, and regular eye exams. Recognizable signs and symptoms include blurry vision, difficulty seeing at night, sensitivity to light, halos around lights, faded colors, double vision, and frequent changes in eyeglass prescription. Etiological factors encompass age, genetics, environmental exposures (e.g., ultraviolet [UV] radiation,), medical conditions (e.g., diabetes, obesity, high blood pressure), eye injuries, certain medications (e.g., corticosteroids, diuretics), and nutritional deficiencies [9].

Scaevola taccada, commonly known as beach naupaka, is a flowering plant belonging to the *Goodeniaceae* family [10]. Native to tropical and subtropical regions of the Pacific and Indian Oceans [10]. The plant displays shiny, dark green leaves and produces distinctive white, fan-shaped flowers with a purple or blue tinge. *S. taccada* holds significance in traditional medicine, where it is employed to treat skin infections, burns, and inflammation [10]. *S. taccada* contains antioxidants that can help protect the body against oxidative stress and damage from free radicals [11].

METHODS

Collection and authentication of plant

The plant *S. taccada* was procured from the medicinal herbal garden of RK University the authentication of the plant was done by Professor Vaibhavi Savaliya, Associate Professor, School of Pharmacy, RK University, Rajkot, Gujarat, India.

Extraction

Once the plant dried, the plant material was collected and crushed.

In this procedure, a round-bottom flask, connected to a Soxhlet extractor and condenser on the heating mantle, is used to receive the solvent (methanol).

The thimble, placed inside the Soxhlet extractor, is filled with crushed plant material. Glass wool is employed to lag the sidearm.

The solvent is heated by heating the mantle, causing it to evaporate as it passes through the apparatus to the condenser. The condensate is collected in the reservoir where the thimble is held, and the cycle restarts as the solvent re-enters the siphon and is poured back into the flask. The entire operation is expected to take 48 h to complete (Fig. 1).

After the procedure concludes, a condensation evaporator should be utilized to evaporate the methanol, resulting in a small yield of the extracted substance (Fig. 2).

Preliminary phytoconstituents screening test

Test for alkaloid

The extract was added to dilute HCl and filter it. And filtrate was collected. Add a few drops of Dragendorff's reagent to the filtrate solution, orange precipitate was produced.

Test for flavonoids

Ammonia test: To the alcoholic solution of 1 g of drug sample, when filter paper dipped and after that exposed to ammonia vapor, the appearance of the yellow spot on the filter paper indicates the presence of flavonoids.

Test for protein

Millon's Test: Extract solution + 2 mL of Millon's reagent (Mercuric Nitrate in nitric acid containing traces of nitrous acid) white precipitate Appear which turns red upon gentle heating.

Test for carbohydrate

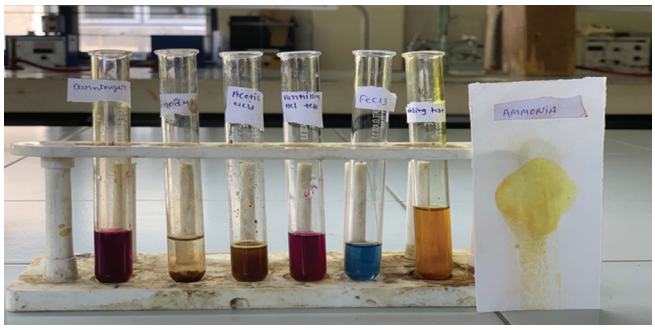
Molisch's Test: The aqueous extract of the powdered leaf when treated with an Alcoholic solution of α-naphthol in the presence of sulfuric acid. Purple color indicates the presence of carbohydrates.

Test for tannins

Ferric chloride test: Extract solution was giving blue-green color Precipitate with $FeCl_3$.

Test for glycoside

Legal test: The substance was dissolved in pyridine, sodium nitroprusside solution Was added to it and made alkaline. A pink or red color indicates the presence of cardiac Glycosides.



Ex vivo phase

In the *ex vivo* and *in vitro* phase study, artificial aqueous humor was meticulously prepared and maintained at room temperature with a pH of 7.8, incorporating streptomycin to prevent microbiological contamination. Goat eyeballs obtained fresh from the Slaughterhouse, were transported in an icebox containing synthetic aqueous humor. The goat eye lens was surgically extracted by making an incision in the eyeballs, separating the cornea and pupil. The isolated goat eye lens underwent 72-h incubation at room temperature in the pre-prepared artificial aqueous humor, maintaining a pH of 7.8. Subsequently, *in vitro* cataract induction was initiated using glucose (0.1 M) to simulate the pathological conditions associated with cataract development. At higher concentrations, the sorbitol pathway metabolizes glucose in the lens, leading to the accumulation of polyol and encouraging oxidative damage and overhydration, ultimately resulting in cataract formation.

Fig. 1: Soxhlet extraction

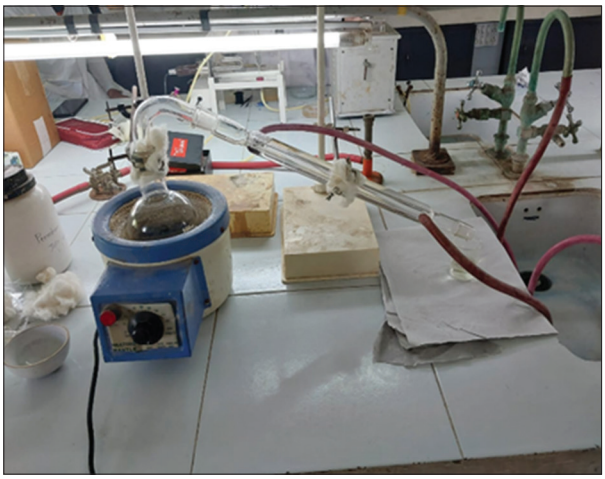


Fig. 2: Condensation process

The Normal Control group was exposed to artificial aqueous humor with a glucose content of 0.1 M, serving as the baseline condition for the lens culture.

The Disease Control group, representing a pathological condition, received artificial aqueous humor with an elevated glucose content of 1 M, intended to induce specific alterations in the lens environment conducive to cataract development.

The Standard Control group was subjected to artificial aqueous humor containing Gallic acid at a concentration of 500 µg/mL, in addition to 1 M glucose. Gallic acid, a known antioxidant, was introduced to explore its potential protective effects against the detrimental impact of high glucose levels on the lenses.

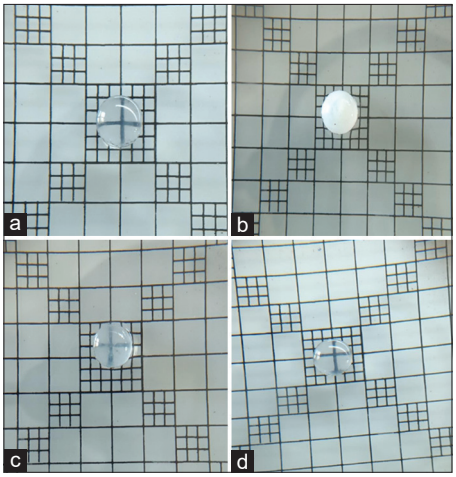


Fig. 3: (a) Normal control group, (b) Disease control group, (c) Standard control group, (d) MEST (500 µg/mL)

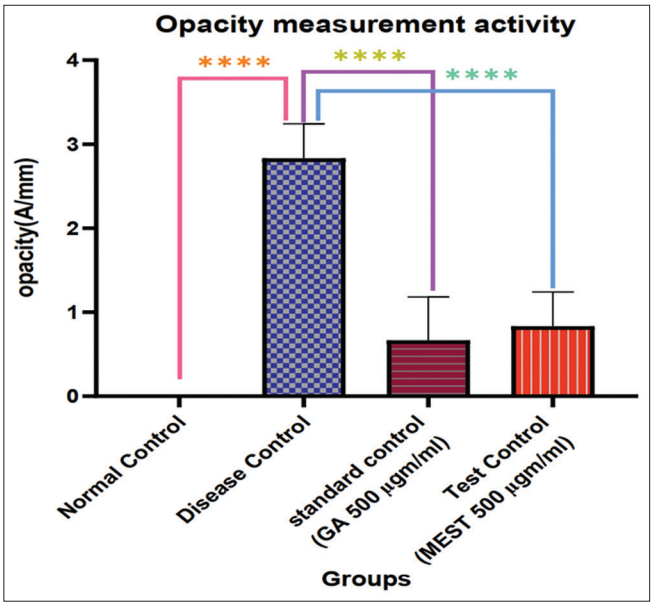


Fig. 4: *In vitro* study of effect of methanolic extract of *Scaevola taccada* in glucose induced cataract on goat lens in opacity activity. *Indicates significant from Model Control at p<0.05

The Test Drug group was exposed to a combination treatment, involving artificial aqueous humor with a glucose content of 1 M and an extract at a concentration of 500 µg/mL for 3 days.

In the morphological and photographic evaluation of lenses post a 72-h incubation period, a systematic approach was adopted. Placing the lenses on graph paper allowed for a detailed assessment of opacity and size. Opacity was graded based on the clarity of square line patterns beneath the lenses, categorized as absence/clear visibility, slight opacity, diffuse opacity, and diffuse to moderate diffusor opacity. This standardized method not only provided insights into the lenses' morphological characteristics but also ensured the reliability and reproducibility of the evaluation process.

Malondialdehyde (MDA) level estimation

In the estimation of MDA levels, a byproduct of lipid oxidation, the principle of this method involves the reaction of one molecule of MDA with two molecules of thiobarbituric acid (TBA) in a moderately acidic environment, resulting in the formation of a hazy chromogen. The

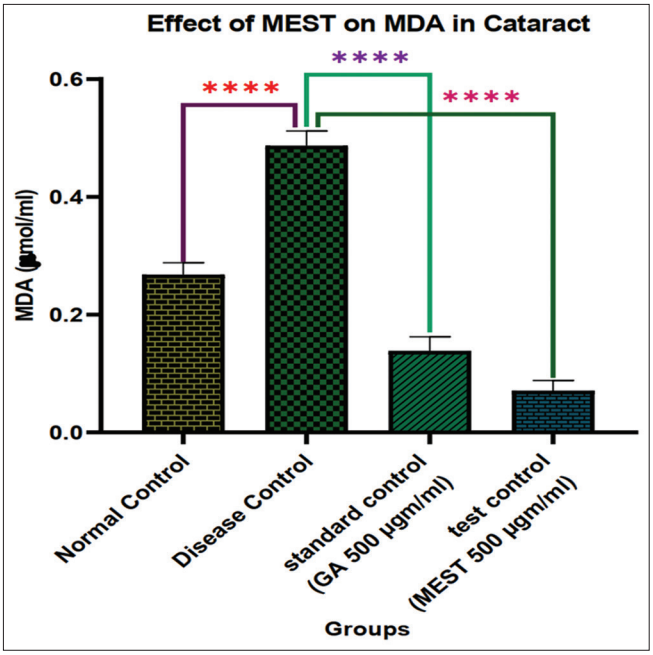


Fig. 5: *In vitro* study of effect of methanolic extract of *Scaevola taccada* in glucose induced cataract on goat lens in malondialdehyde level. *Indicates significant from Model Control at p<0.05

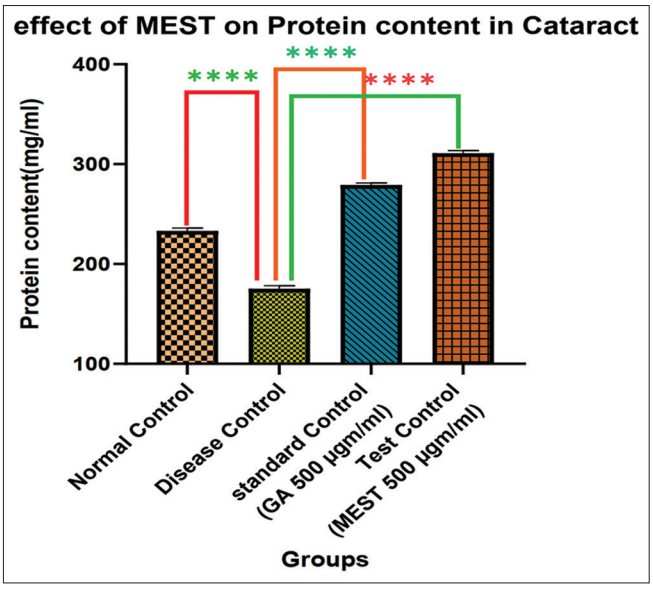


Fig. 6: *In vitro* study of effect of methanolic extract of *Scaevola taccada* in glucose induced cataract on goat lens for protein estimation

absorbance of the generated hazy solution was measured using a UV Spectrophotometer at 532 nm and 600 nm at intervals of 180–200 s. Following a 72-h incubation period, lenses were homogenized in Tris's buffer (23 M) with a pH of 7.8 and containing 0.25×10^{3} EDTA using a sonicator and stirrer. The homogenate was then adjusted to 10% w/v and centrifuged at 10,000 rpm/min for 1 h, with the supernatant collected for further analysis. The reagents used include TBA (0.067% Tris hydrochloride, pH 7) and Trichloroacetic acid (20%). The procedure involves the preparation of blank and test solutions, followed by the measurement of absorbance at 532 nm and 600 nm at 3-min intervals. The calculation is based on the absorbance values obtained at these wavelengths.

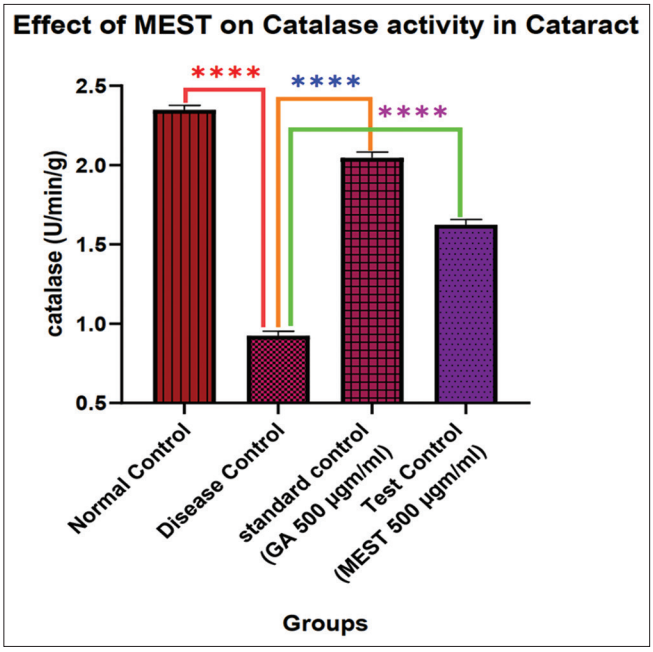


Fig. 7: *In vitro* study of effect of methanolic extract of *Scaevola taccada* in glucose induced cataract on goat lens for catalase activity

Estimation of catalase activity

In the assessment of catalase activity, the Aebi approach was employed, focusing on the enzyme's role in safeguarding cells against oxidative damage caused by unstable reactive oxygen radicals. The method utilized the characteristic increase in absorption of hydrogen peroxide (H_2O_2) with decreasing wavelength, as elucidated by Aebi. The breakdown of H_2O_2, a process integral to catalase function, was directly correlated with a reduction in absorbance at 240 nm, allowing for the measurement of changes in absorbance over time as an indicator of catalase activity.

For the experimental procedure, a pH 7 phosphate buffer was prepared by combining potassium dihydrogen phosphate and disodium phosphate in a 1:1.5 (v/v) ratio, resulting in a solution that was crucial for maintaining the optimal conditions for catalase activity. A reaction mixture was then prepared by combining 1 mL of phosphate buffer, 1 mL of hydrogen peroxide (30 mol/L), and 2 mL of the homogenate solution. The absorbance of this mixture was measured, and water was used as the blank for reference.

The catalase activity was quantified by tracking the rate of H_2O_2 breakdown, observed through the reduction in UV absorbance at 240 nm. This systematic approach allowed for a precise evaluation of catalase efficiency in the homogenate solution, providing valuable insights into the enzymatic defense mechanism against oxidative stress. These findings contribute to a deeper understanding of cellular responses to oxidative challenges, offering potential implications for therapeutic interventions targeting oxidative damage.

Effect of MEST on protein content in cataract

The investigation into the impact of MEST on protein content in cataracts involves a chemical principle where copper ions, under the influence of a basic medium, are reduced and interact with protein peptide bonds. The synthesis of hetero-polymolybdenum blue, imparting a blue color to the solution, occurs when the folincateachu reagent is reduced with the assistance of a peptide complex link. The reagents employed include a mixture of 0.1 N NaOH with 2% sodium carbonate, a solution comprising water and sodium potassium tartrate, another solution of water and $CuSO_4$, and a combination of Folin-Phenol (2 N) and water. In addition, an albumin standard at 1 mg/mL serves as a reference.

In the experimental procedure, 0.2 mL of lens homogenate for the test solution and 0.2 mL of albumin solution for the protein standard were separately placed in test tubes, with distilled water adjusting the volume to 1 mL. Solution 1, comprising a mixture of A, B, and C, was added to each test tube and incubated for 10 min. Following incubation, 0.5 mL of Solution 2 (D) was added to all test tubes, and another 30-min incubation followed. Absorbance was measured at 660 nm in a UV spectrophotometer, using distilled water as a blank.

The calculation involved determining the quantity of protein in the sample from a standard graph, reported in mg/di after the absorbance standard graph was plotted. This meticulous procedure allowed for the quantitative assessment of protein content in the lens homogenate, shedding light on the potential influence of MEST on cataract-related protein alterations.

Statistical data analysis

Statistical analysis is performed to check the significance of the data.

1. Analysis of variance (ANOVA): To see the variability within all the groups.
2. Tuckey's test: For the same purpose mentioned in the above test.
3. p-value, Degree of freedom, Standard deviation, etc.
4. p=0.05 was regarded statistically significant, while p=0.001 was considered very significant.

The statistical analysis was performed by ANOVA followed by Tuckey's test using PRISM software.

RESULTS

Opacity activity

In this model, we used Gallic acid as a standard drug, glucose as disease control, and water as control, and test drug with a dose of 500 µg/mL.

Dose of test drug: *S. taccada* (500 µg/mL).

It was studied that *S. taccada* has the ability to improve in glucose induced cataractogenesis in goat lens. After treatment with MEST at a dose of 500 µg/mL resulted a decrease in Opacity activity level as compared to the disease control group (2.83±0.21) with std. and test control (0.67±0.27) and (0.83±0.21), respectively.

MDA activity

In this model, we used Gallic acid as a standard drug, glucose as disease control, and water as control, and test drug with a dose of 500 µg/ml.

Dose of test drug: *S. taccada* (500 µg/mL).

It was studied that *S. taccada* has the ability to improve in glucose induced cataractogenesis in goat lens. After treatment with MEST at a dose of 500 µg/mL resulted decrease in MDA level as compared to the disease control group with (0.126±0.006) and (0.1751±0.010), respectively.

Total protein estimation activity

In this model, we used Gallic acid as a standard drug, glucose as disease control, and water as control, and test drug with a dose of 500 µg/mL.

Dose of test drug: *S. taccada* (500 µg/mL).

It was studied that *S. taccada* has the ability to improve in glucose induced cataractogenesis in goat lens. After treatment with MEST at a dose of 500 µg/mL resulted increase in Total Protein level as compared to a standard control, disease control group with (306.73±1.59), (285.93±1.3) and (246±1.47), respectively.

Estimation of catalase activity

In this model, we used Gallic acid as a standard drug, glucose as disease control, and water as control, and test drug with a dose of 500 µg/mL.
Dose of test drug: *S. taccada* (500 µg/mL).

It was studied that *S. taccada* has the ability to improve in glucose induced cataractogenesis in goat lens. After treatment with MEST at a dose of 500 µg/mL resulted increase in Catalase level as compared to standard control with (1.82±0.014) and (1.61±0.015), respectively.

DISCUSSION

Anti-cataract agents are compounds that have been shown to prevent or delay the formation of cataracts, which are cloudy areas that form in the lens of the eye and can cause vision problems. These agents can play a vital role in the prevention and treatment of cataracts. As a part of the study, we evaluated the potential of our plant (*S. taccada*) as an anti-cataract.

Some anti-oxidants and flavonoids as well as carotenoids may help in reducing the cataract formation. In addition to that, *S. taccada* is also having the same phytoconstituents.

To evaluate the anti-cataract activity, we performed several models to check the potential of our plant and these models are as follows:

1. MDA level estimation on goat lens.
2. Total protein estimation.
3. Catalase activity.

MDA level estimation on goat lens

MDA is a toxic compound and if the MDA level is high, it indicates the high level of oxidative stress and leads to cataract formation. By measuring the MDA level, it can be evaluated the effectiveness of anti-oxidants as anti-cataract. Anti-oxidants can scavenge the free radicals and decrease the MDA level.

Hence, as a part of the study, we measured MDA level on the goat lens with normal control, disease control, and standard control as well as with our test drug (500 µg/mL). This data revealed that the test drug has a low MDA level as compared to Disease control. Hence, we can say that it may prevent cataract formation and act as an anti-cataract.

Total protein estimation

Protein is crucial for the transparency and function of the lens of the eye. Oxidative stress cause damages to lens protein and leads to cataract formation. Anti-oxidants reduce oxidative stress to prevent cataract formation. Therefore, it is important to measure the total protein in all test groups to evaluate the anti-cataract activity As a part of the study, we measured the total protein level on the goat lens with normal control, disease control, standard control as well as with our test drug (500µg/ml). This data revealed that the total protein level was increased significantly in our test drug in compared to disease control and standard control. Based on that we can conclude that the test drug (*S. taccada*) may work as an anti-cataract.

Catalase activity

Catalase is an enzyme that is having role of protection of lens from oxidative damage. If catalase activity is low then it is more susceptible to oxidative damage and leads to cataract formation. Anti-oxidants play a crucial role to enhance the defense system and in the prevention of cataracts. Hence, it is important to measure the catalase activity to evaluate the potential of our test drug as an anti-cataract.

Hence, we performed a model to check the catalase activity. Catalase activity data revealed that the test drug is having high catalase activity as compared to the standard control group. It shows that the anti-oxidants of our plant enhance the defense system of a lens and help in the prevention of cataracts.

CONCLUSION

The study carried out clearly revealed that *S. taccada* (Bhadrax) has the potential for anti-cataract activity. This hypothesis was supported by the following findings MDA level estimation, total protein level, and catalase activity. From the above findings, we can that *S. taccada* (Bhadrax) has the potential for anticataract activity.

AUTHORS CONTRIBUTION

The experimental design, guidance, supervision, and review of the research were carried out by Meet Galathiya, Savan Chhatrola, and Bhargav Kamani conducted the experimental work, developed and optimized the formulations, interpreted the results, and drafted the manuscript. Tejas Ganatra has read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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