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Original Article

ANTICATARACT POTENTIAL OF BARLERIA PRIONITIS: IN VIVO STUDY

MOHAMMED ATIF*[1], SHAIK ABDUL RAHMAN[2], MOHAMMED IBRAHIM AHMED[1], SYED BAQUER MAHMOOD[1], MOHAMMED AZHARUDDIN[3]

***[1]Dawood Villa, Bazar Ghat, Nampally Hyderabad 500004, [2]Omer Colony, Hafeez baba nagar, Hyderabad, 500005, Telangana, [3]Bada bazar, Ahmed Nagar, Hyderabad 500028, [4]Darushifa, Hyderabad-500024, [5]Moosarambagh, Hyderabad, Telangana-500036**
Email: atif_mohammed2001@yahoo.com

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ABSTRACT

Objective: The present study was formulated in order to evaluate the Anticataract potential of *Barleria prioni*tis using Selenite and Galactose induced cataract models.

Methods: *Barleria prionitis* (Family-Acanthaceae), used in mythical system possessing antioxidant activity was evaluated for its Anticataract potential. The ability of *Barleria prionitis* to tweak the biochemical parameters was explored in these studies. Selenite cataract was incited in 10 days old pups by subcutaneous infusion of sodium Selenite (25 moles/kg of body weight). The rats in the test gathering were infused with *Barleria prionitis* (200 and 400 mg/kg body weight, p. o.) 4 h before the Selenite administration. The rate of cataract was watched when the rats initially opened their eyes on 16th day. Galactose cataract was actuated in rats by sustaining 30% Galactose in the eating regimen. Rats in the test gathering were sustained orally with 200 and 400 mg/kg of *Barleria prionitis* every day and rats in the control gathering got just vehicle. Cataract stages were evaluated at normal interims.

Results: In this study, morphological assessment demonstrated that Selenite treated gathering demonstrates increased opacities when contrasted with normal gathering. A fall in the glutathione level and a climb in the Malondialdehyde levels were seen in control rather than normal lenses. Administration of *Barleria prionitis* significantly restored the glutathione and Malondialdehyde levels. SOD, Catalase and Glutathione S transferase levels were significantly restored to normal levels ($p<0.05$ and $p<0.01$ respectively). Oral administration of *Barleria prionitis* significantly delayed the onset and progression of cataract in Selenite as well as Galactose induced cataract.

Conclusion: The Anticataract potential is evident from the slit lamp microscopic images. It can be said that *Barleria prionitis* significantly reversed the cataract parameters by virtue of its antioxidant potential, which may be useful for further molecular studies to determine the exact mechanism for its Anticataract potential.

Keywords: *Barleria prionitis*, Oxidative Stress, Antioxidant, Vitamin E.

INTRODUCTION

Herbal medicines have as of late pulled in much consideration as optional prescription valuable for treatment and anticipation of way of life related disorder [1]. Nonetheless, moderately almost no information is accessible about their mode of activity and security. The most punctual recorded utilization of herbal cures originates from Hippocrates, who bolstered utilization of straightforward plants, for example, garlic, neem [2].

Cataract, a visual disability creating unsettling influence in lens transparency, happens primarily because of opacification or optical dysfunction of crystalline lens. Cataract is the significant reason for visual deficiency on the planet. It contributes to half of sightlessness around the world. More than 17 million individuals are visually impaired due to Cataract. Give or take 25% of the populaces beyond 65 years old and around half beyond 80 years old have genuine loss of vision on account of cataract. In India alone the yearly rate of cataract visual deficiency is around 3.8 million. Visual disability in cataract results from mistiness or light scrambling created regularly by the creation of huge protein aggregates in the lens [3]. Different components, for example, sunshine, diet, diabetes, hypertension, renal failure, drying out, and oxidation of lens proteins and peroxidation of lipids ascribe to the era of lens opacities in the older individual [4].

In spite of the fact that, cataract is a multi-factorial infection, oxidative systems through generation of reactive oxygen radicals are accepted to assume a vital part in the dynamic decay of vision and shaping of cataract[5]. Reactive oxygen species (ROS, for example, hydrogen peroxide, superoxide radical, singlet oxygen and hydroxyl radical are proposed to contribute to this cataract process. Endogenous safeguard systems through rummaging of ROS by antioxidant enzymes like superoxide dismutase, glutathione peroxidase, catalase and glutathione-S-transferase, at the same time ensure the lens from oxidative damage [6]. Biological antioxidants such as pyruvate [7] and nutritional antioxidants like ascorbate, Vitamin E and carotenoids were found to delay the development of experimental cataract [8].

Shortly, not very many meds, eye drops, exercise or glasses are accessible to cure or avert cataracts[9]. Anyway, the main accessible treatment for the disease is the surgical extraction of the cataractous lens emulated by supplanting with a synthetic insert. Surgical treatment is prescribed when a cataract advances to the point that it weakens visual capacity. In view of the sufficient proof that oxidative stress assumes a part in component of cataractogenesis, there has been an expanding enthusiasm toward the improvement of suitable Anticataract prevention agent results of plant origin that could be powerful in postponing or preventing the formation of cataract. Lately, an extraordinary attention has been laid on investigating the likelihood of utilizing our natural assets to defer the onset and progression of cataract. A number of medicinal plants and their formulations are accounted for to have antioxidant properties and offer protection against cataract one of them being Barleria prionitis which has been accounted for its Antioxidant potential and as there is no particular reports on the Anticataract activity of the Barleria prionitis. Therefore, the present study was attempted to assess the Anticataract capability of *Barleria prionitis* ethanolic concentrate in selenite and Galactose affected test model of cataract.

MATERIAL AND METHODS

Collection and authentication of the plant material

Leaves of *Barleria prionitis* commonly known as Vajradanti (Hindi), Kundan (Tamil), Porcupine Flower (English)[10], the plant was

checked for data in www. plantlist. org with the following statement (This name is accepted name of a species in the genus Barleria (family Acanthaceae). The record derives from WCSP (in review) which reports it as an accepted name with original publication details*: Sp. Pl. 636 1753*. The plant parts like leaves are used as an Antioxidant, anti-inflammatory. The leaves of *Barleria prionitis* were procured from Bhimavaram. West Godavari, Andhra Pradesh, India supplied by Ilas Challapatti during the month of March 2013.

The collected plants were positively identified by Botanical Survey of India, Hyderabad with a Ref. **No. BSI/DRC/2013-14/Tech./455**.

Experimental animals

9-days–old rat pups (Wistar strain) were used in selenite induced Cataract. The pups were housed with parents in large spacious cages and the parents were given food (standard pellet diet) and water ad libitum. The animal room was well ventilated. For Galactose induced cataract healthy Wistar Albino rats weighing about (120-160 gm) of either sex were obtained from animal house. The animals were maintain under standard condition i. e., housed in polypropylene cages and maintained at a temperature 27 ± 2^{0}C, relative humidity 65 ± 10% under 12 hour light and dark cycle. The animals were acclimatized for 10 days under laboratory condition before carrying out the experiments. The animal house approved by the Committee for the Purpose of Control and Supervision on Experimental Animals (CPCSEA)-Registration number – **(1330/AC/10/CPCSEA)**. The study was carried out after the approval by the institutional animal ethical committee (IACE) [11].

Chemicals

All the chemicals were Analytical grade. Sodium Selenite, Galactose, Vitamin E and Ethanol were obtained from SD chemicals, Hyderabad.

Method of preparation of extract

The collected leaves were washed thoroughly under running water, cut into smaller pieces and air dried for eight days. Then the dried leaves were coarsely powdered using the grinder and were continuous extracted in a soxhlet apparatus at 30^{0}C with 2500 ml ethanol. The extract was filtered through a fine muslin cloth and evaporated under reduced pressure by the rotary evaporator. The obtained extracts were stored in amber colored glass bottle for further processing [12].

Preliminary phytochemical screening

The solution of the methanolic extract was prepared using distilled water and subjected to preliminary phytochemical screening. Test for common phytochemicals were carried out by standard methods described in practical pharmacognosy by Kokate, Khandelwal and Trease and Evans [13-15].

Determination of acute toxicity (OECD guideline 423)

The acute toxicity for ethanolic extract of leaves of *Barleria prionitis* was determined in albino rats following OECD guideline 423, maintained under standard conditions [11].

Evaluation of anticataract potential

Selenite induced Cataract [16]

The 10 days old wistar rat pups were used for inducing Cataract. A normal, negative control, reference standard and 2 test groups were used each comprising of 6 pups.

Group I: Received only saline (Normal) 5 ml/kg by p. o

Group II: Normal Saline + Selenite 25 µmole/kg body weight (Negative Control) subcutaneous on day 1.

Group III: Vitamin E 50 mg/kg b. w., per oral + single dose of Selenite 25 µmole/kg b. w. subcutaneous on day 1.

Group IV: *Barleria prionitis* ethanolic extract Test dose 1 + single dose of Selenite 25 µmole/kg b. w. subcutaneous on day 1.

Group V: *Barleria prionitis* ethanolic extract Test dose 2 + single dose of Selenite 25 µmole/kg b. w. subcutaneous on day 1.

Each rat pup in group II received a single subcutaneous injection of 25 µmole/kg body weight of sodium selenite, group III received vitamin E 50mg/kg body weight p. o, and groups IV and V received test extracts at a dose of 200 and 400 mg/kg body weight.

Above treatment was continued for one week, on the last day a slit lamp examination was performed on each eye of rat pup for morphological evaluation of any lenticular opacification.

Mydriasis was achieved prior examination using ophthalmic solution containing tropicamide with phenylepherine, one drop of solution was instilled every 30 min for 2 hour. After 2 hour eyes were viewed by a slit lamp.

Galactose induced cataract [17]

Wistar rats of either sex weighing 100 to 150 Gms were used, divided into 5 groups each comprising of 6 rats each.

Group I: Normal saline 5 ml/kg body weight.

Group II: Normal saline + 30% Galactose diet (Negative control).

Group III: Vitamin E 50 mg/kg body weight, p. o. + 30% Galactose diet.

Group IV: *Barleria prionitis* ethanolic extract Test dose 1 + 30% Galactose diet.

Group V: *Barleria prionitis* ethanolic extract Test dose 2 + 30% Galactose diet.

Treatment was started a week prior to Galactose administration. All the groups were treated for 30 days. Cataract was examined on the 30th day under slit lamp. On the next day lenses were removed from the eyes of all the rodents for estimation of Total protein, Catalase, Malondialdehyde (MDA), Glutathione, Glutathione peroxidase and Superoxide dismutase (SOD).

Grading stages of cataract

At the end of experimental period the degree of lenticular opacification was graded and photographed in both selenite and Galactose induced cataract. The degree of opacification was graded as follows,

- Stage I- Lenses similar to normal lenses
- Stage II- Lenses showing faint peripheral opacity
- Stage III- Nuclear cataract
- Stage IV- Mature opacity involving entire lens

Statistical analysis

Results were expressed as Mean ± SEM. Statistical analysis were performed with Graph pad prism software using one way Analysis of Variance followed by Dunnett's *t*-test.

P values were considered significant when *P<0.05, **P<0.01, ***P<0.001 when the test and standard were compared with the untreated groups [11].

RESULTS

Preliminary phytochemical analysis

The phytochemical screening of ethanolic extract of *Barleria prionitis* leaves revealed the presence of Alkaloids, flavonoids, saponins, tannins and Phenolic compounds.

Acute toxicity studies

The acute toxicity studies of *Barleria prionitis* ethanolic leaves extract was carried out as per OECD guideline no. 423. There was no gross evidence of any abnormality observed up to a period of 4-6 hrs or mortality up to a period of 24 hrs at the maximum tolerated dose level of 2000 mg/kg body weight p. o. Further pharmacological screening were carried out with two dose ranges i. e. 200 mg/kg b. w. p. o. and 400 mg/kg b. w. p. o.

Effect of *Barleria prionitis* ethanolic extract on Selenite induced cataract

Effect on GSH, MDA levels and on enzyme activity

Administration of selenite resulted in a significant decrease of GSH levels in negative control group (31%) in comparison to normal control group (7.68±0.27). Supplementation with Vitamin E and test extract BPEE 400 mg/kg significantly restored the GSH levels ($p<0.001$ and $p<0.01$ respectively). Malondialdehyde (MDA) levels showed a drastic rise of 52% in negative control group in comparison to normal group. Vitamin E and BPEE 400mg/kg b. w, p. o significantly reversed the increased MDA levels (Table 1).

Glutathione peroxidase, Superoxide dismutase, Catalase and Total protein levels were significantly lowered. A significant restoration of the activities was found as a result of treatment with Vitamin E and BPEE 400mg/kg b. w, p. o.

Table 1: Effect of *Barleria prionitis* on GSH, MDA levels and on Enzyme Activity

Groups	GSH (µmol/mg of protein) Mean± SEM	MDA (nmol/gm) Mean± SEM	SOD (IU/g) Mean± SEM	GPx (µmol/mg of protein) Mean ± SEM	Catalase (µmol/mg) Mean± SEM	Total Protein (mg/ml)
Group I (Normal)	7.68±0.27	61.16±0.11	3.14 ± 0.64	42.72±6.03	7.48±0.04	933.3 ± 0.92
Group II (Negative control)	2.39±0.22[a]	93.2±0.23[a]	1.21± 0.27[a]	26.55±0.18[a]	3.83±0.13[a]	226.9 ± 1.07[a]
Group III (Vitamin E)	7.17±0.31***	65.6±0.65**	2.87 ±0.12**	40.71±0.12**	6.65±0.10**	816.61±5.06***
Group IV (BPEE 200mg/kg)	4.59±0.19*	89.4±0.49*	2.21 ± 0.53*	31.91±0.09*	4.20±0.20	555.3±1.05**
Group V (BPEE 400mg/kg)	5.96±0.17**	72.1±1.2**	2.71 ± 0.06**	31.91±0.09**	5.92±0.12*	653.7±5.02**

All the values are expressed as Mean ± SEM (n = 6); a compared to normal group ($p<0.001$), significances values are ***$p<0.001$, **$p<0.01$, *$p<0.05$ (versus Negative control group)

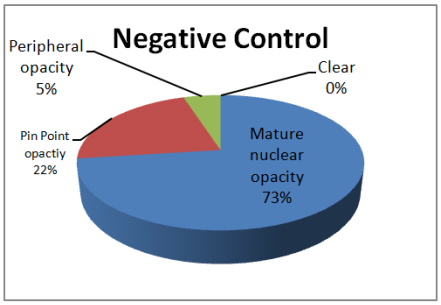
Effect of Selenite on lens Morphology

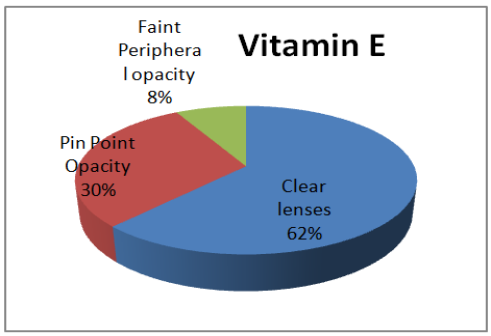
Rat pups when injected with a dose of sodium selenite subcutaneously resulted in the development of nuclear opacity in 100% of pups in the control group on 16th postnatal day. 73% showed mature nuclear opacity whereas 22% revealed pin point opacity and 5% peripheral opacity Graph 1. Vitamin E treated group showed a significant decrease of cataract, 62% had clear lenses, 30% pin point opacity and only 8% had Faint peripheral opacity. Supplementation with BPEE 400mg/kg, b. w, p. o also decreased the extent of opacities, 22% were clear, 58% had faint peripheral opacity, 12% had peripheral opacity and only 8% exhibited nuclear opacity.

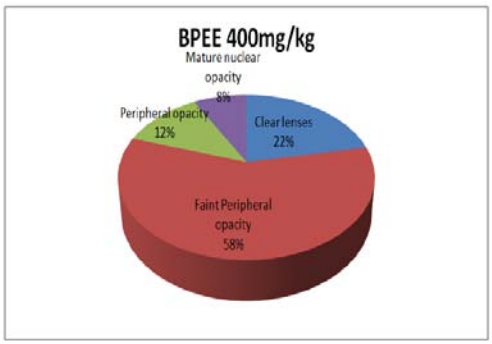
Effect of *Barleria prionitis* ethanolic extract on Galactose induced cataract

Treatment with Galactose brought about a noteworthy decline of GSH levels in negative control bunch (41.3%) in correlation to ordinary control assembles (10.3±0.39) table 2. Supplementation with Vitamin E and test concentrate BPEE 400 mg/kg fundamentally restored the GSH levels ($p<0.001$ and $p<0.01$ separately). Malondialdehyde (MDA) levels demonstrated an uncommon ascent of 60.69% in negative control group in examination to normal gathering. Vitamin E and BPEE 400mg/kg b. w, p. o altogether turned around the expanded MDA levels.

Glutathione peroxidase, Superoxide dismutase, Catalase and Total protein levels were fundamentally brought down. A noteworthy rebuilding of the activities was found as an aftereffect of treatment with Vitamin E and BPEE 400mg/kg b. w, p. o.







Graph 1: Effect of Vitamin E and *Barleria prionitis* ethanolic extract (BPEE) on selenite cataract in rat pups.

Effect of Galactose on lens Morphology

Cataract onset in Galactose fed rats in control group was observed on the 7th day. All eyes in the control group revealed cataractogenic changes. In BPEE 400mg/kg 32% exhibited normal eyes on day 7 whereas supplementation in Vitamin E group rats had 75% clear lenses. By the end of experimental period i. e. on 30th day all the lenses in negative control group developed mature nuclear opacity, whereas Vitamin E treated group showed 52% clear lenses with 25% having stage II opacity. 20% lenses of BPEE 400mg/kg b. w, p. o exhibited clear nature, while 60% were stage II and the remaining 20% had fair nuclear opacity and faint peripheral opacity (Graph 2).

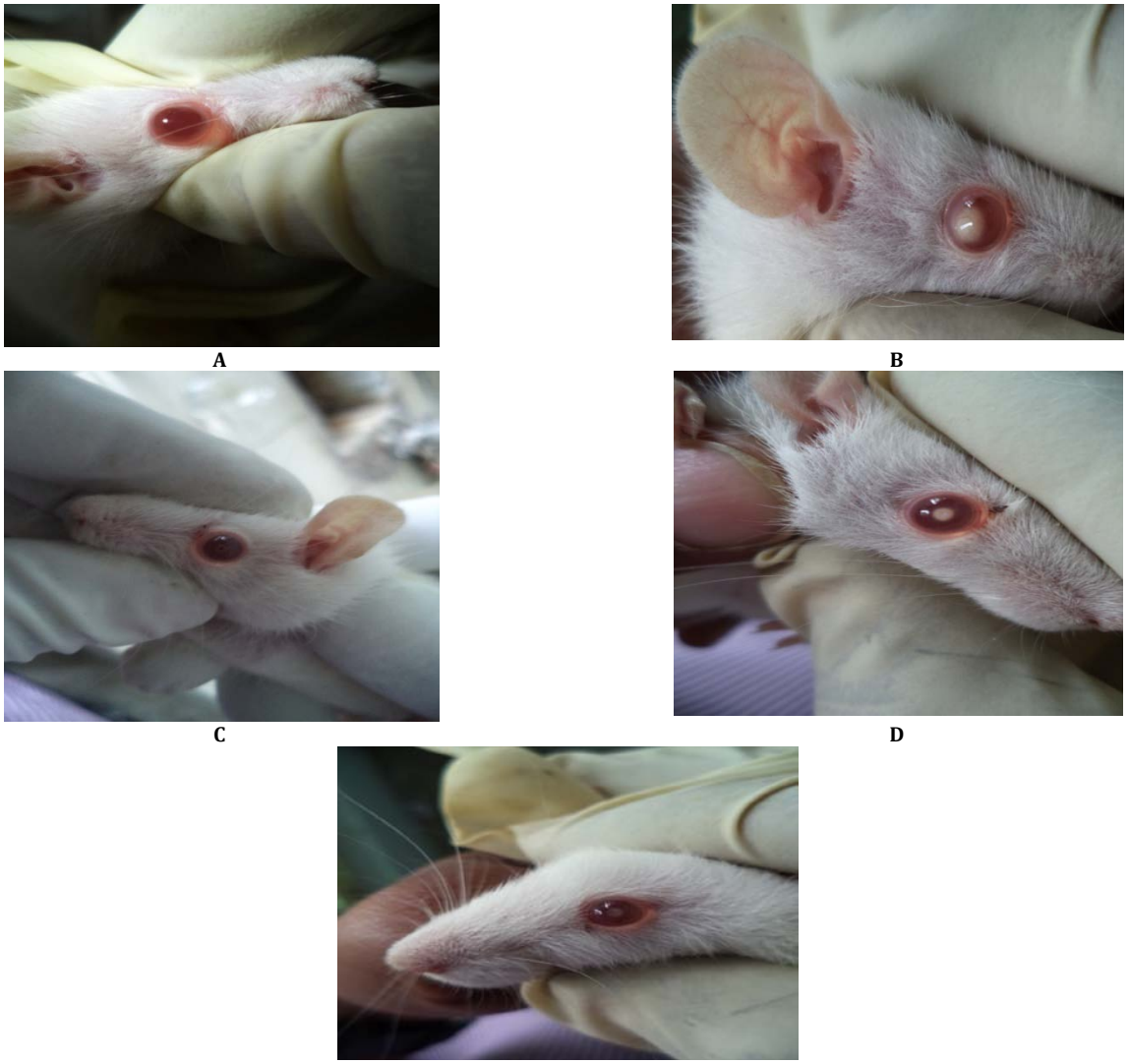


Fig. 1: Effect of Selenite on Lens Morphology a) Normal lens when treated with normal saline b) Negative Control group exhibiting Stage IV Mature nuclear opacity when treated with Selenite c) Vitamin E showing Stage II faint peripheral opacity d) BPEE 200mg/kg + Selenite having stage III opacity e) BPEE 400mg/kg + Selenite exhibiting stage II peripheral opacity

Table 2: Effect on GSH, MDA levels and on Enzyme Activity in *Barleria prionitis* ethanolic extract (Galactose induced Cataract)

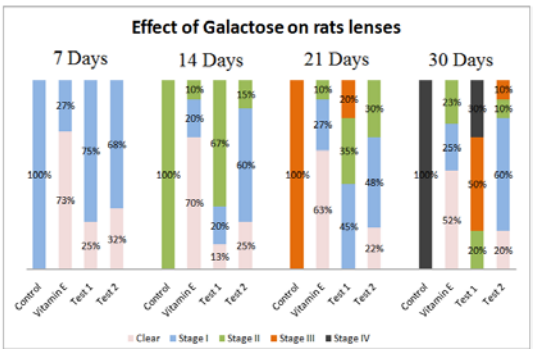
Groups	GSH (µmol/mg of protein) Mean ± SEM	MDA (nmol/gm) Mean ± SEM	SOD (IU/g) Mean ± SEM	GPx (µmol/mg of protein) Mean ± SEM	Catalase (µmol/mg) Mean ± SEM	Total protein (mg/ml)
Group I (Normal)	10.33±0.30	51.27±1.19	2.22±0.29	31.39±0.63	6.21±0.38	868.12±13.17
Group II (Negative control)	4.27±0.35[a]	82.39±0.37[a]	0.36±0.11[a]	12.81±0.18[a]	2.17±0.42[a]	212.37±4.36[a]
Group III (Vitamin E)	9.39±0.32***	53.56±0.47**	2.01±0.37**	29.28±0.12***	5.96±0.27***	838.97±10.88***
Group IV (BPEE 200mg/kg)	6.57±0.29*	71.32±0.32*	1.17±0.15*	20.77±0.56*	3.11±0.17*	489.47±12.17*
Group V (BPEE 400mg/kg)	7.33±0.31**	60.31±0.19**	1.86±0.19**	26.91±0.9**	4.16±0.23**	686.11±9.27**

All the values are expressed as Mean ± SEM (n = 6); a compared to normal group ($p<0.001$), significances values are ***$p<0.001$, **$p<0.01$, *$p<0.05$ (versus Negative control group)

DISCUSSION

Cataract is a significant cause for visual impairment and of serious visual impedance prompting two-sided difficulty seeing in an expected 20 million individuals around the world. In developing nations, most of the visual impairment is brought about by Cataracts. Pharmacological treatment against human cataract has so far not been accomplished. Subsequently, surgery to uproot the opacified lens is the main viable treatment for the cataract. The difficulties are to anticipate or postponement cataract formation furthermore to treat in the long run on the off chance that it happens. The exact component of cataract formation is still not clear. There are studies to examine the component of Cataractogenesis utilizing distinctive models of cataract and to target basic steps to stop this procedure. Among different models, the selenite induced and Galactose induced cataract model are a standout amongst the most generally utilized test models.[18]

In the present study one of the locally available plant *Barleria prionitis* was selected. Cataract was induced by sodium selenite and Galactose which are commonly used models in screening of Anticataract drugs. Glutathione (GSH), Malondialdehyde (MDA) levels and the antioxidant enzyme activity was evaluated for determining the Anticataract potential of the plant. Lens morphology was checked in both the models in order to determine the effectiveness Vitamin E and the test extracts.



Graph 2: Effect of Vitamin E and *Barleria prionitis* ethanolic extract (BPEE) on Galactose induced cataract in rats

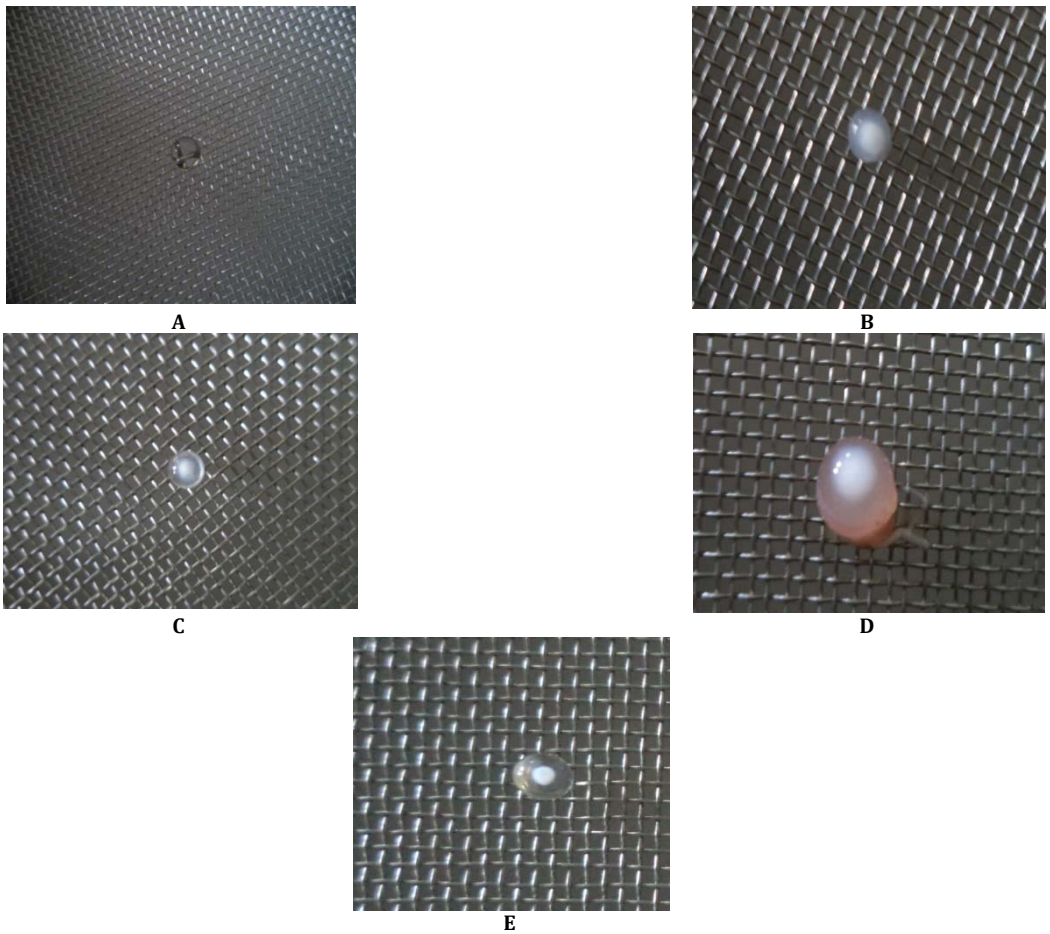


Fig. 2: Effect of Galactose on Lens Morphology a) Normal lens when treated with normal saline b) Negative Control group exhibiting Stage IV Mature nuclear opacity when treated with Galactose c) Vitamin E showing Stage II faint peripheral opacity d) BPEE 200mg/kg + Galactose having stage III opacity e) BPEE 400mg/kg + Galactose exhibiting stage II peripheral opacity

A few biochemical courses of action happen amid creation of selenite cataract. These incorporate modified epithelial digestion system, calcium amassing, calpain-impelled proteolysis, stage move, and cytoskeleton loss, yet the exact mode of selenite activity is still not self-evident. Selenite appears to uncover its impact on lens by inciting oxidative stress in the lens tissue. Oxidation of protein and nonprotein sulfhydryl bunches is a result of selenite administration; this prompts to unsettling influence of the electrolytic offset. The intracellular calcium level builds, which actuates the calcium dependent protease calpain. Intracellular proteins are partially hydrolyzed by Calpain, particularly β-crystallin. This is joined by a reduction in exercises of the cell reinforcement catalysts, for example, SOD, glutathione peroxidase (Gpx), CAT, and GSH [16]. The biochemical estimations showed decrease in the antioxidant enzyme levels such as SOD, Gpx, Catalase and total proteins and also a decrease in Glutathione levels in both models. But these enzyme

levels were significantly restored to normal levels in Standard group (Vitamin E) and BPEE 400mg/kg b. w, p. o ($p<0.001$). Malondialdehyde levels showed a jump in negative control group of selenite as well as Galactose induced cataract. Treatment with standard and the test extracts reversed the levels of MDA in both the models.

Prevention or retarding oxidative damage to sulfhydryl groups in lens epithelium by BPEE 400mg/kg may be the mechanism behind the Anticataract potential of the plant. A decrease in the onset and a delay in progression of cataract in rats feeding on BPEE 400mg/kg were seen in Galactose fed rats.

Treatment with test extracts in selenite and Galactose induced cataract reduced the number of rats with mature nuclear opacity (stage IV) there by presenting a clear evidence of Anticataract potential of the plant.

This effect may be associated with maintaining the antioxidant enzyme activities and decreased MDA levels. Our preliminary results are encouraging, but further molecular studies are needed to clarify the exact mechanism behind the anticataractogenic potential of the *Barleria prionitis*.

CONCLUSION

The phytochemical assessment indicated the presence of Alkaloids, flavonoids, saponins, tannins and Phenolic compounds. The acute toxicity studies of BPEE were carried out and no gross evidence of abnormalities or mortality were found in the rats even at a maximum tolerated dose level of 2000mg/kg b. w, p. o.

The study on the assessment of the Anticataract capability of *Barleria prionitis* in exploratory animals showed that it tweaks the antioxidant parameters. It attenuates the selenite instigated cataract and deferrals the onset and progression of Galactose induced cataract, these biochemical changes reiterate the important role of oxidative stress in Cataractogenesis where the *Barleria prionitis* ethanolic extract might be valuable for cataract treatment.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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