

## Pharmacological screening of aqueous extract of *Sesbania grandiflora* for anti-glaucomic activity in Rabbits

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The present work was designed to investigate the antiglaucoma activity of *Sesbania grandiflora* (SG) aqueous leaf extract against alpha chymotrypsin induced experimental glaucoma in rabbits. The experimental increase in IOP was achieved using alpha chymotrypsin induced glaucoma model. Once the IOP was increased significantly, aqueous leaf extract of SG and standard were given topically every day into the right eye of rabbits. IOP was measured by Schiottz indentation tonometer on every alternate day till a significant reduction of IOP was observed. The results were compared with standard 0.1% brinzolamide. A significant increase in IOP was observed on the 7<sup>th</sup> day after inducing glaucoma. Significant reduction of IOP was observed on the 6<sup>th</sup> day after giving plant extract when compared with the standard. The results show that the leaf extract showed significant oculohypotensive activity and this effect was comparable to the standard brinzolamide. Further investigation into the mechanism of action and isolation of compounds which are responsible for antiglaucoma activity is to be established.

**Keywords:** Alphachymotrypsin, Brinzolamide, Intraocular pressure (IOP), Schiottz tonometer.

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### Introduction

Glaucoma is described as a group of eye conditions leading to the interruption of visual information from the eye to the brain<sup>1</sup>. It is the second most important cause of blindness after cataract<sup>2,3</sup>. There are many risk factors for glaucoma but the most important is the rise in intraocular pressure which causes damage to the optic nerve<sup>4,5</sup>. Although surgical options exist, medical management to control IOP is the mainstay of the treatment<sup>6</sup>. There are different synthetic medications available in the market but their cost, side effects and contraindications limit their use in patients. Since many ages, Botanical compounds were used as a cure for various diseases and ailments. They have a very long history of medical use. 74% of today's modern drugs that are used directly in traditional medicine have their origin from the natural compounds<sup>7</sup>. Classical texts of Ayurveda have attributed wide-ranging therapeutic indications of this selected herb. *Sesbania grandiflora* popularly known as Agasthya leaves are known to possess wide therapeutic applications. It is used as an analgesic and CNS depressant<sup>8</sup>, in smoke-related diseases<sup>9</sup>,

antioxidant and anti urolithiatic<sup>10</sup>, cardioprotective<sup>11</sup>, protective effect on kidneys<sup>12</sup>, including eye diseases<sup>13-15</sup>. The plant was reported to contain Alkaloids, Glycoside, Tannins, Carbohydrates, etc<sup>16-18</sup>. However, relatively little scientific information is presented on the usage of this drug in treating ocular diseases such as glaucoma. Hence, in the present study, we studied the oculohypotensive activity of this plant in reducing IOP.

### Materials and Methods

#### Chemicals

Alpha chymotrypsin (Sisco Research Laboratories, Hyderabad, India), brinzolamide, indomethacin, midazolam, phenobarbitol and xylocaine are commercial samples procured in Apollo pharmacy, Hyderabad.

#### Animals

New Zealand rabbits of either sex weighing 2 kgs were used. The animals were treated in accordance with the institutional guidelines (CPCSEA approval no. 1358/ac/10) to make use of animals in research. The animals were acclimatized for a period of 2 weeks, *ad libitum* food and water was provided and 12 h light/dark cycle was maintained. After two weeks of habituation in the animal house facility, the animals were trained to accept tonometry.

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#### Collection & authentication of plant material

The leaves of *Sesbania grandiflora* Linn. (SG) belonging to the family Fabaceae were collected in the month of April 2015 from our native town Pithapuram, East Godavari Dist., A.P. The leaves were authenticated (authentication number is 901) by the Dr. K. Mahdava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, A.P., India. The voucher specimen of the plant was preserved in the herbarium, Plant taxonomy lab of Sri Padmavathi Mahila Viswavidyalayam, Tirupati, A.P., for reference.

#### Extraction

Fresh leaves of SG were collected and dried in an oven at 50 °C until it attained constant weight and pulverized to coarse powder. The powdered material was subjected to maceration process for 3 days followed by Soxhlet extraction for 3 hours. The extract was filtered and concentrated under reduced pressure using a rotary evaporator<sup>19</sup>. Two doses were used for the study i.e. 200 and 400 mg/kg body wt. The results were compared with standard, 0.1% brinzolamide.

#### Experimental methodology

The rabbits were divided into four groups of 5 rabbits each (n=4). All the rabbits were given sterile isotonic saline solution (0.05 mL) containing 150 units of alpha-chymotrypsin was irrigated through the cannula into the posterior chamber of the right eye. The left eye was treated as a control for all the animals<sup>20</sup>.

Group I served as a toxicant group; Group II was given with standard 0.1% brinzolamide eye drops; Group III & IV were given orally with two doses of plant extract 200 and 400 mg/kg body wt. of SG respectively.

#### Induction of glaucoma

To prevent the immediate onset of inflammation, rabbits were pretreated with 10 mg/kg i.p. indomethacin and to abolish any nystagmus they were slightly anesthetised with phenobarbital. The right eye was anesthetised topically with 4% xylocaine eye drops. A 30-gauge cannula was inserted into the anterior chamber near the limbus and directed to the posterior chamber through the pupil. Through this cannula, a sterile isotonic saline solution (0.05 mL) containing 150 units of alpha-chymotrypsin was irrigated into the posterior chamber. Care was taken to avoid the injection of any enzyme into the corneal

stroma. Cannula is then carefully removed without significant loss of aqueous humor. Only the right eye was injected with alpha chymotrysin, the left eye was treated as control<sup>20</sup>.

#### Measurement of IOP

IOP was measured with Schiotz indentation tonometer after instillation of 2% lignocaine eye drops. After significant elevation of IOP rabbits were given with standard drug and plant extract. Every day at the same time standard and plant extract were given orally to the respective groups. On every alternate day, IOP was measured in both eyes till it reduces to control value. The results were compared with standard, 0.1% brinzolamide.

#### Statistical analysis

The variation between the toxicant and treated groups were analyzed using two way ANOVA. The differences were considered significant if P was at least <0.05.

#### Results

Glaucoma was induced by 150 units of alpha chymotrypsin and antiglaucoma activity was established by measuring IOP. Measurement of IOP is the usual procedure in the diagnosis and management of glaucoma. The basal IOP was measured for all the rabbits in both the eyes before giving alpha chymotrypsin. Statistically, no significant difference was observed in the IOP of the left and right eyes in all the groups. After inducing glaucoma with alpha chymotrypsin IOP was measured every day. It was increased and almost double the basal IOP on the 7<sup>th</sup> day. When compared with basal IOP, induced IOP significantly increased up to 108.5% in group I, 101.81% in group II, 94.38 % in group III, and 101.80% in group IV.

After giving plant extract and standard on every alternate day IOP was measured. IOP reduced gradually in all the rabbits till 6<sup>th</sup> day of the study. Insignificant IOP reduction was observed after 2<sup>nd</sup>-day post treatment whereas on the 4<sup>th</sup> day and 6<sup>th</sup> day post treatment there was a significant reduction in IOP was observed in all the rabbits given with SG and also with the standard when compared with rabbits of the toxicant group. There was a drastic drop in IOP was observed from 4<sup>th</sup> day to 6<sup>th</sup> day post treatment.

In Group II rabbits treated with standard, 0.1 % brinzolamide peak IOP reduction of 4.8% on the 2<sup>nd</sup> day, 23% on the 4<sup>th</sup> day and 46.1% on the 6<sup>th</sup> day were

observed. It was a significant reduction when compared with the toxicant group.

In Group III rabbits given with SG with a dose of 200 mg/kg, IOP reduction was found to be 9.7% on the 2<sup>nd</sup> day, 14% on the 4<sup>th</sup> day and 39.9% on the 6<sup>th</sup> day.

Rabbits were given with 400 mg/kg of SG i.e. in Group IV, IOP reduction was found to be 12.3% on the 2<sup>nd</sup> day, 14.7% on the 4<sup>th</sup> day and 41.5% on the 6<sup>th</sup> day.

### Discussion

Glaucoma does not represent a single pathological entity. It consists of a long group of disorders with widely differing clinical features. It is characterized by progressive optic nerve cupping and visual field loss. It is caused by increased fluid pressure within the eye compressing the nerves at the back, which can lead to blindness if not treated<sup>21</sup>. Through early detection, diagnosis and treatment can help to preserve the vision. Although several risk factors are involved in glaucoma, IOP is the main factor<sup>22</sup>. Increase in IOP leads to mechanical compression and optic nerve damage<sup>23</sup>. The drugs used to reduce IOP may increase the aqueous humour drainage or decrease the production of aqueous humour<sup>24</sup>. Beta blockers,  $\alpha$  adrenergic receptor agonists, carbonic anhydrase inhibitors are popular drugs used for the treatment of glaucoma.

From ancient times, drugs from a natural source are used in curing various diseases including ocular problems<sup>25</sup>. But there is very little scientific information is available on the use of these drugs in curing ocular diseases. These drugs produce lesser side effects when compared with synthetic drugs.

The parameter used in the present study was a chronic model of glaucoma induced by alpha

chymotrypsin. Alpha chymotrypsin is a proteolytic enzyme secreted by the pancreas. Alpha chymotrypsin was injected intravitreally into rabbits elevates the ocular pressure by 5 to more than 25 mm Hg within 2 to 3 days. The proteolytic action of alpha chymotrypsin on zonular material leads to the formation of debris of the tissue, which blocks the aqueous outflow channels, closed angle, peripheral anterior synechiae and leads to inflammatory reactions in trabecular meshwork hence elevates the IOP<sup>26</sup>.

Carbonic anhydrase is found in the form of isoenzyme in many tissues of the body including the eye. It is secreted in the ciliary epithelial cells. It is involved in the reversible reaction that is hydration of carbon dioxide and the dehydration of carbonic acid. Inhibition of this enzyme in the ciliary cells decreases aqueous humour secretion, possibly by slowing the formation of bicarbonate ions which follows a reduction in sodium and fluid transport<sup>27</sup>. The end result is a decrease in intraocular pressure. Brinzolamide is a CA inhibitor used topically or orally in treating glaucoma.

Intragroup comparisons were summarized in Table 1. Before inducing glaucoma basal IOP was measured. No statistical difference was observed between the left and right eyes of all the rabbits. After inducing glaucoma into the right eye, there was a statistical difference was observed on the 7<sup>th</sup> day. After giving the drugs, every alternate day IOP was measured. On the 2<sup>nd</sup> and 4<sup>th</sup> day of post drug treatment, a statistically significant difference was observed. On the 6<sup>th</sup> day of post drug treatment statistically no significant difference was observed between left and right eye since IOP reduced to control value.

Table 1 — Intragroup Comparison of IOP Changes & (% Reduction of IOP) (n=4)

Groups	Basal IOP		Induced IOP		IOP on 2 <sup>nd</sup> Day		IOP on 4 <sup>th</sup> Day		IOP on 6 <sup>th</sup> Day	
	Left eye	Right eye	Left eye	Right eye	Left eye	Right eye	Left eye	Right eye	Left eye	Right eye
Group I	18.68± 0.419	18.86± 0.311	18.45± 0.623	39.24± 1.159 (108.50)	18.56± 0.379	38.14± 1.209	19.13± 0.470	38.91± 0.496	18.24± 0.856	38.35± 0.655
Group II	19.22± 0.249	18.75± 0.344	19.73± 0.847	37.84± 0.578 (101.81)	19.59± 0.482	36.02± 1.091 (4.8)	19.67± 0.383	29.15± 0.501 (23)	19.39± 0.440	20.39± 0.558 (46.1)
Group III	18.49± 0.226	19.36± 0.492	18.64± 0.822	36.32± 1.325 (94.38)	18.59± 0.559	32.79± 0.906 (9.7)*	18.38± 0.430	31.23± 0.907 (14)*	18.22± 1.000	21.82± 0.055 (39.9)*
Group IV	16.42± 0.531	17.39± 0.729	18.38± 0.951	34.39± 0.675 (101.80)	18.88± 0.231	30.17± 0.917 (12.3)*	18.47± 0.451	29.33± 0.834 (14.7)*	20.58± 0.278	20.12± 0.457 (41.5)*

Intergroup comparisons were summarized in Fig. 1 & 2. When the effect of the plant extract and standard were studied on the reduction of IOP, there was statistical significant variation was observed between the toxicant group and the treatment group. Whereas statistically no significant difference was observed between the treatment group and disease standard group. This shows the drug is equally effective in reducing IOP as the percentage of reduction of IOP was similar.

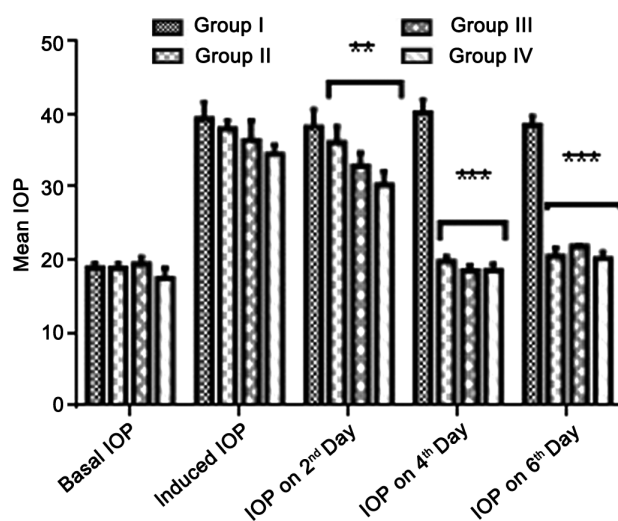


Fig. 1 — Intergroup comparison of % reduction of IOP in comparison with induced IOP (Right eye) (n=4). The results are Mean±S.E. of four parallel measurements (p < 0.05). The results were compared with two way ANOVA. The asterisk (\*) denotes that the data are significantly different from the standard group.

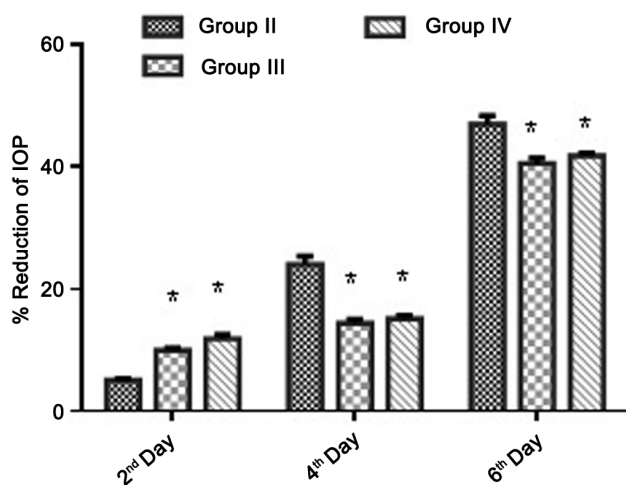


Fig. 2 — Intergroup comparison of IOP changes in comparison with induced IOP (Right eye) (n=4). The results are Mean±S.E. of four parallel measurements (p < 0.05). The results were compared with two way ANOVA. The asterisk (\*) denotes that the data was significantly different from the toxicant group.

## Conclusion

It can be concluded from the above shown results that the aqueous leaf extract of *Sesbania grandiflora* showed significant oculohypotensive activity and this effect was comparable to the standard brinzolamide and the reduction in IOP of this plant might be due to the same mechanism like carbonic anhydrase inhibitors i.e. reducing the production of aqueous humour by direct antagonistic activity or by some other mechanism which is to be established. Hence further studies are to be done to establish the mechanism of action of this plant in reducing IOP and also to know the active constituents responsible for this action.

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