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OCULOHYPOTENSIVE EFFECTS OF *FOENICULUM VULGARE* IN EXPERIMENTAL MODELS OF GLAUCOMA

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Abstract : Purpose : Evaluation of oculohypotensive activity of single drop application of aqueous extract of *Foeniculum vulgare* in experimental models of glaucoma. **Methods:** *The evaluation of oculohypotensive activity of Foeniculum vulgare was done* in rabbits with normal intraocular pressure (IOP) and with experimentally elevated IOP. The experimental increase in IOP was achieved using water loading and steroid induced glaucoma models.

Results : The aqueous seed extract of *Foeniculum vulgare* exhibited 17.49, 21.16 and 22.03% reduction of intraocular pressure (IOP) in normotensive rabbits at 0.3%, 0.6% and 1.2% (w/v) concentrations respectively. The 0.6% concentration was further evaluated in acute and chronic models of glaucoma. A maximum mean difference of 31.20% was observed between vehicle treated and extract treated eyes in water loading model while a maximum mean IOP lowering of 31.29% was observed in steroid induced model of glaucoma.

Conclusions : The aqueous extract of *Foeniculum vulgare* possesses significant oculohypotensive activity, which was found to be comparable to that of timolol. Further investigations into the mechanism of action, possible toxicity and human clinical trials are warranted before the *Foeniculum vulgare* finds place in the arsenal of antiglaucoma drugs prescribed by physicians.

Key words : *Foeniculum vulgare* oculohypotensive experimental models glaucoma

INTRODUCTION

Glaucoma is a progressive optic neuropathy and elevated intraocular pressure (IOP) is considered the major risk factor. Although surgical options exist, medical management to control IOP is the mainstay of the treatment. Timolol, a beta-blocker has long been used for reduction of IOP in glaucoma. It reduces IOP by suppressing the aqueous humor formation. Topical application of timolol to normal rabbit eyes has been shown to reduce IOP by 20–30% (1). However, topical application of timolol to oculohypertensive rabbit eyes is known to reduce IOP to a greater extent. Although

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a number of other drugs are also available, the cost, side effects and contraindications limit their use in all patients. The drugs from natural sources if found effective in reducing IOP without toxic manifestations in glaucomatous eyes, may prove to be an important and inexpensive antiglaucoma agent.

Foeniculum vulgare is a well-known medicinal plant and belongs to the family Umbelliferae. Aqueous extract of *Foeniculum vulgare* seeds was shown to possess antihypertensive properties in spontaneously hypertensive rats, possibly because of its diuretic activity (2). *Foeniculum vulgare* has also shown antioxidant, nitric oxide scavenging (3) and anticholinesterase activity (4) in various *in vivo* and *in vitro* experiments. Keeping in view the above-mentioned properties of *Foeniculum vulgare* the present study was designed to evaluate antiglaucoma activity of its aqueous seed extract.

METHODS

Animals

All procedures in this study complied with ARVO statement for the use of animals in research as well as with the local regulatory and ethical requirements. New-Zealand white rabbits of either sex, weighing 2.5–3.5 kg were used. Animals were housed under standard laboratory conditions with 12-hour cycles of light and dark (light from 6 a.m. to 6 p.m.) and were provided with normal pellet diet and tap water ad libitum. All animals were examined and those found normal on general and ophthalmic examination were included in the study. After one week of habituation in animal house facility the animals were trained to accept tonometry.

Test drugs

The plant, *Foeniculum vulgare*, was collected from the Haryana state of India and species was identified by comparison with herbarium specimen and HPTLC finger printing. Extract (FVE) was prepared by soaking the seeds in water followed by filtration and drying of filtrate in vacuum oven at 60°C. This yielded a dry extract, which was powdered and stored in sealed containers at 4°C. The yield of the extract as a percentage weight of the starting plant material was 8:1. FVE was dissolved in 0.25% autoclaved hydroxypropyl methylcellulose (HPMC) so as to give a concentration of 0.3%, 0.6% and 1.2% (w/v). HPMC was used to increase the corneal residence time. This was followed by filtration using 0.22 (µm Millipore filter. The filtrate was kept in sterile amber coloured vials before use. The timolol maleate 0.25% was used as a reference standard.

Estimation of IOP

The IOP was estimated using Non-Contact Tonometer (NCT) Nidek-2000, Japan. The technique of using NCT in rabbits has already been described by the authors (5).

Experimental models

The evaluation of IOP lowering activity of test drug was carried out in normotensive rabbits and in rabbits with experimental increase in IOP. The experimental increase

in IOP was achieved using water loading and topical instillation of steroid. In water loading model IOP elevation was achieved in unanaesthetized rabbits according to the modification of a previously described procedure (6, 7). The conscious rabbits were rapidly administered with 70 ml/kg of tap water through an orogastric tube. This induced an acute rise in IOP lasting for at least 2 hours. In steroid induced model the IOP elevation was achieved in young rabbits by topical instillation of 10 µl of prednisolone 1% eye drops in both eyes, twice a day for a period of 40 days. The IOP estimations were repeated twice a week during the period of steroid instillations. Topical instillation of glucocorticoids has been shown to cause elevated IOP in human (8, 9) and rabbits (10, 11, 12). A 31.58% increase from baseline could be achieved at the end of 40 days period however, a mortality of 20% was also observed during steroid treatment.

Study design

The study was carried out in two steps.

Step 1: Normotensive rabbits were randomly divided into 3 groups of 12 rabbits each. On the day of experiment baseline IOP was estimated at 8.30 a.m. The rabbits in first group were instilled with 50 µl of 0.3% FVE in one of the randomly chosen eye while the other eye was treated with 50 µl of 0.25% hydroxypropyl methylcellulose. Groups 2 and 3 received 50 µl of 0.6% and 1.2% FVE respectively in one of the eye while the other eye received an equal volume of vehicle as in group 1. IOP was estimated at an interval of one hour for a total of 8 hours.

Step 2: The dose of FVE showing maximum peak IOP reduction from baseline in step 1 was further evaluated in water loading and steroid induced models of glaucoma. For evaluation in water loading model rabbits were divided into two groups of 12 animals each. The baseline IOP was estimated after overnight fasting at 8.30 a.m. The first group was instilled with 50 µl of timolol 0.25% in one of the randomly chosen eye and 50 µl of vehicle in the other eye. The second group received 50 µl of FVE in one of the randomly selected eye and same volume of vehicle in the other eye. One hour after the drug/vehicle instillation, rabbits were administered with tap water (70 ml/kg) through an orogastric tube. This was followed by IOP estimations at an interval of 15 minutes for a total of 120 minutes.

For evaluation in steroid induced model of glaucoma the rabbits pretreated with prednisolone 1% were randomly divided in three groups of 6 animals (12 eyes) each. On the day of experiment the baseline IOP was estimated. First group was now instilled with 50 µl of vehicle in both eyes while the second and third groups received same volumes of timolol 0.25% and FVE (0.6%) respectively in both eyes. This was followed by IOP estimations at hourly intervals for a total duration of 6 hours.

Statistical analysis

One-way ANOVA was used for determining the statistical significance of the differences between groups at probability level of 95%.

RESULTS

Normotensive model

In normal rabbits treated unilaterally with FVE 0.3%, the IOP declined gradually and at 3 hours post treatment the mean percent IOP reduction in drug treated eye was 18.50% from baseline as compared to 1.01% in vehicle treated control eye. The maximum mean IOP reduction in normotensive rabbit eyes treated with 0.6% FVE was 21.83% while the same with 1.2% FVE was 23.63% as compared to 0.67 and 1.60% in control eyes respectively. This maximum IOP reduction was observed at 3 hours post treatment. Change in IOP after treatment with FVE 0.3% was significantly less than that after treatment with FVE 0.6 and 1.2% (P<0.05) (Fig. 1). There was no significant difference in IOP reduction between groups treated with FVE 0.6 and 1.2% and therefore FVE 0.6% was chosen for further evaluation in water loading and steroid induced models.

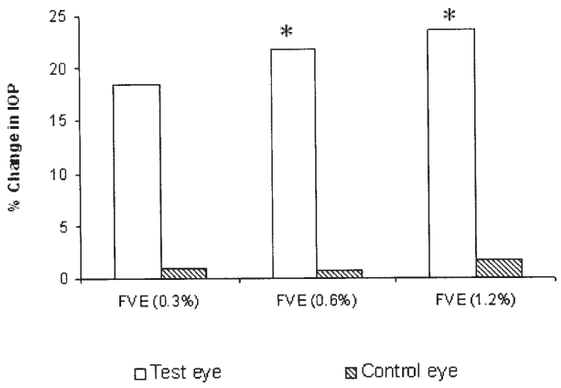

Fig. 1 : Oculohypotensive activity of *Foeniculum vulgare* in normotensive rabbit. Topical instillation of 0.3%, 0.6% and 1.2% FVE to normotensive rabbit eye (n=12) resulted in a 18.50, 21.83 and 23.63% reduction in IOP at 3 hours post treatment. The IOP reduction was significantly greater in all three dose groups as compared to IOP changes in corresponding control eye (P<0.0001). The IOP reduction was significantly lower in 0.3% FVE treated group as compared to 0.6% and 1.2% FVE treated groups (P<0.05) however no significant difference was observed between 0.6% and 1.2% FVE treated groups. *P<0.05 as compared to FVE 0.3%.

Water loading model

The unilateral treatment with FVE as well as timolol resulted in significant protection against the rise in IOP in response to water loading. The rise in IOP in FVE 0.6% treated eyes was significantly less as compared vehicle treated eyes from 15 minutes to 105 minutes after water loading (P<0.001). A maximum mean difference of 31.20% in IOP rise was observed between control and treated eyes in FVE treated group at 60 minutes after water loading. In timolol treated group a maximum mean difference of 31.40% in IOP rise was observed between control and treated eyes at 45 minutes after water loading with a significant difference persisting throughout the experimental period (P<0.001) (Table I, Fig. 2). A significantly higher protection

TABLE I : Change in IOP in water loaded rabbits following instillation with FVE 0.6% and timolol moleate 0.25%.

Water loading model			*Steroid induced model*		
Time after loading (minutes)	*FVE (0.6%)*	*Timolol (0.25%)*	*Time post drug instillation (Hours)*	*FVE (0.6%)*	*Timolol (0.25%)*
15	–14.94	–22.6	1	–7.22	–22.98
30	–21.38	–28.3	2	–21.05	–31.80
45	–30.14	–31.4	3	–30.73	–33.76
60	–31.20	–24.3	4	–28.41	–27.85
75	–21.86	–23.8	5	–18.39	–23.71
90	–20.07	–22.9	6	–4.49	–20.56
120	–8.52	–12.1	8	–1.87	–7.57

All value are group mean. (n=12 for each group).

against the rise in IOP was observed in timolol treated group during first 30 minutes post water loading as compared to FVE treated group ($P<0.05$).

Steroid induced model

The mean peak IOP reduction in timolol and FVE treated groups was 33.76 and 30.73% respectively, 3 hours post treatment. Significant IOP reduction in FVE treated group was observed for 1–6 hours post treatment ($P<0.001$ from 2–5 hours and $P<0.01$ at 1 and 6 hours). The timolol treated group showed significant IOP reduction from 1–8 hours post treatment ($P<0.001$ up to 6 hours and $P<0.05$ at 8 hours). (Table I, Fig. 3).

DISCUSSION AND CONCLUSIONS

The present study evaluates the IOP lowering effect of single drop application of FVE in rabbits with normal IOP as well as in rabbits with experimental increase in IOP. The experimental increase in IOP was achieved by water loading and topical steroid application. The water loading model with acute rise in IOP in this experiment closely resembles the clinical situation with rapid rise in IOP whereas the steroid induced model closely resembles primary open angle glaucoma as marked similarities have been noted between the aqueous outflow obstruction, optic nerve cupping and visual field defects associated with glucocorticoid induced glaucoma and those found in POAG (13, 14, 15). For steroid treatment both eyes were treated with prednisolone instead of one of the randomly chosen eye because 20% mortality indicated significant amount of systemic absorption and significant systemic absorption is expected to give rise to changes in the untreated eye as well, making it difficult to detect the actual rise in steroid treated eye when compared with untreated eye as control. In addition as per our experience the mortality observed after unilateral steroid treatment is no different from bilateral steroid treatment. Therefore, it was considered appropriate to randomize the bilaterally steroid treated animals into different groups. This also helped in making use of the minimum possible number of animals to give statistically significant results. Pharmacological utility of these models was well demonstrated by time course of IOP lowering activity of timolol and FVE.

Single drop application of all three concentrations of FVE (0.3, 0.6 and 1.2%) resulted in a significant fall in IOP in rabbits with normal intraocular pressure ($P<0.001$). FVE 0.6 and 1.2% treated groups showed no significant difference in mean peak IOP reduction but the IOP reduction was significantly higher in both groups as compared to FVE 0.3% treated group. Further evaluation of 0.6% FVE in water loading model showed a significant difference (31.20%) in IOP rise between control and treated eyes in FVE treated group ($P<0.001$). This protection by FVE against the rise in IOP in response to water loading was comparable to that caused by timolol (31.40%). The maximum difference in IOP between control and treated eyes in timolol treated group was observed at 45 minutes after water loading while the same was at 60 minutes after water loading in FVE treated group indicating a comparatively slower onset of action in the later group. A significantly higher protection against the rise in IOP in timolol treated group ($P<0.05$) as compared to FVE group for initial 1st hour can also be attributed to slower onset of

action of FVE. Similar differences in the time course of IOP lowering effects of timolol and FVE were also observed in steroid induced model. No significant difference was observed in peak IOP reduction of 33.76% in timolol group and 30.73% in FVE group at 3 hours post treatment in steroid model. The total duration of significant IOP reduction was 8 hours for timolol and 6 hours for FVE and a significantly higher IOP reduction with timolol during first and last 2 hours of observation period. It can be concluded from the above-discussed results that the *Foeniculum vulgare* aqueous extract shows significant oculohypotensive activity and the maximum IOP lowering effect of FVE is comparable to that of timolol. However, the onset of action of FVE is slower and duration of action is shorter than that of timolol. The mechanism of action of IOP lowering activity of FVE might be related to its anticholinesterase activity as has been shown by earlier experiments (4). Investigations to further explore possible mechanism of action of FVE are in progress. Investigations with isolated active ingredients are desired. However, the possibility of synergistic effects among known active principles and unknown compounds in the plant extracts should always be considered.

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