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Lowering of intraocular pressure by topical application of *Daucus carota* seed extract in rabbits

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In normotensive rabbits topical application of *Daucus carota* seed extract at the concentration of 0.3, 0.6 and 1.2% resulted in mean IOP reduction of 19.33. 23.20 and 25.61% respectively from baseline. As no significant difference was observed between the change in IOP in 0.6 and 1.2% extract treated groups, 0.6% concentration was chosen for further evaluation in rabbits with experimentally elevated IOP. In water loaded rabbits, maximum mean IOP reduction with 0.6% extract was 29.39%, which was comparable to pilocarpine. In steroid pretreated rabbits, maximum mean IOP reduction was 30.27% from baseline, which was significantly higher than pilocarpine. The extract showed a comparatively slower onset of action however, the duration of action was comparable to pilocarpine in all the experimental models.

Keywords: *Daucus carota*, Intraocular pressure, Rabbit

The treatment of ocular diseases using natural remedies has a long history in Ayurveda, Chinese, European and other systems of medicine. *Daucus carota* has long been used to promote ocular health because of its high carotene contents. The plant belongs to family Apiaceae and is commonly known as carrot. Although it has not been traditionally used for the treatment of many ocular diseases, there appears a possibility of its beneficial effects in diseases like glaucoma owing to its traditional uses as an antihypertensive[1] and diuretic agent[2].

Glaucoma is a leading cause of irreversible visual blindness and multiple risk factors are known to be involved in the onset and progression of the disease. In spite of the knowledge of various risk factors involved, modulation of intraocular pressure (IOP) remains the preferred modality of treatment. Experimental drugs which are known to possess antihypertensive, cholinergic and antioxidant properties can also be expected to possess IOP lowering potential. *Daucus carota* has been shown to possess these properties in various experiments. The hypotensive activity of *Daucus carota* was demonstrated by Gilani *et al*. in rats[1]. Increased carrot consumption is also associated with improved antioxidant status[3,4]. The water-soluble fractions from alcoholic seed extract have shown the presence of cholinergic smooth muscle relaxant active principles[5]. The cholinesterase inhibitory activity of *Daucus carota* seed extract has been described earlier[6]. Therefore, it was considered appropriate to evaluate IOP lowering effects of its aqueous seed extract in experimental animal models.

Materials and Methods

All the methods followed in this study were in accordance with the ARVO statement of Use of Animals in Ophthalmic and Vision Research and due permission was taken from the Institutional Animal Ethics Committee.

Animals—Twelve New Zealand white rabbits weighing 2-2.5 kg were used in each group. The animals were maintained under standard laboratory conditions with 12 hr cycle of light and dark and normal pellet diet and tap water was provided *ad libitum*. The animals were allowed to acclimatize in the animal house facility for 1 week before subjecting to tonometry.

Tonometry—IOP estimation of all rabbit eyes was done using NT-2000 (Nidek, Japan) auto Non Contact Tonometer (NCT). The technique of using NCT in rabbits has recently been described by the authors[7].

Extract preparation—Plant *Daucus carota* was procured from the Haryana state of India. The plant

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material was identified by comparison with the herbarium specimen and HPTLC fingerprints of the extract. Aqueous seed extract was prepared by soaking the powdered seeds in water followed by filtration and drying of filtrate using vacuum oven at 60°C. The final yield of the extract was 15:1 as a percentage weight of starting plant material. The dried and powdered extract was dissolved in 0.25% hydroxy propyl methylcellulose (HPMC) followed by filtration using 0.22 μm Millipore filter. As the HPMC is a viscoelastic substance it increases the corneal residence time. The pilocarpine nitrate 2% was used as reference standard.

Experimental models and study design

IOP lowering effect of *Daucus carota* seed extract (DCE) was studied in nornotensive rabbits as well as in rabbits with experimentally elevated IOP. The methods used to achieve experimental elevation of IOP provided both the acute and chronic models of glaucoma. Acute IOP elevation was achieved using water loading model while for chronic IOP elevation, steroid induced glaucoma model was used.

Normotensive rabbits—A group of 12 rabbits with normal IOP was first subjected to IOP estimations over 24 hr, so as to assess the degree of diurnal variations. Thereafter, IOP lowering effects of DCE were studied in normotensive rabbits using three concentrations i.e. 0.3, 0.6 and 1.2% (w/v). Each dose group consisted of 12 rabbits. On the day of experiment, baseline IOP estimations were obtained for both eyes at 9 AM. One of the randomly chosen eyes in all rabbits was considered the test eye and was instilled with 50 μl of the drug solution. The contralateral eye served as control and was instilled with same volume of vehicle (0.25% HPMC). Subsequently, IOP estimations were repeated at 1 hr interval until the baseline IOP was achieved. The concentration of DCE showing maximum efficacy in terms of per cent IOP reduction from baseline was further evaluated in water loading and steroid induced models. The IOP lowering effect of DCE was also compared with that of pilocarpine in animals with experimentally elevated IOP.

Water loading model—Acute rise in IOP was achieved in conscious rabbits by rapid intragastric administration of tap water (70 ml/kg) through an orogastric tube. Rapid gastrointestinal absorption of significant quantity of water leads to plasma hypo-osmolarity, increased aqueous production and elevated IOP[8,9].

To study the rise in IOP, a group of 12 conscious, untreated rabbits was first administered with water (70 ml/kg) through an orogastric tube and IOP estimations were repeated every 0.25 hr for a total duration of 2.00 hr post water loading.

To evaluate the IOP lowering potential of DCE in water loading model, rabbits were first subjected to baseline IOP estimations. Thereafter, one of the randomly chosen eyes of each rabbit was instilled with 50 μl of the test drug (DCE/pilocarpine) and the contralateral eye received the same volume of vehicle. The test drug/vehicle instillation was followed by water loading as described above. The interval between drug administration and water loading was calculated in such a way that time to peak effect as shown in normotensive eyes corresponds to the time of peak IOP elevation as observed in water loaded untreated rabbits. Subsequently, IOP estimations were repeated at the interval of 0.25 hr for 2.00 hr. Both the DCE and pilocarpine treated groups consisted of 12 rabbits each.

Steroid induced model—To induce chronic rise in IOP, a group of 20 young rabbits was bilaterally instilled with 10 μl of prednisolone 1% twice a day for a period of 40 days and IOP estimation was repeated weekly. Another group of 20 rabbits was bilaterally instilled with the same volume of normal saline for the same duration so as to compare and evaluate changes in IOP in steroid treated group. The corticosteroid induced glaucoma is well known in human and steroid induced IOP elevation in rabbits closely resembles the human disease in clinical features as well as the underlying mechanism[10].

Rabbits, after 40 days pretreatment with prednisolone (1%), were subjected to evaluation of IOP lowering effect of DCE and pilocarpine, both groups consisting of 12 rabbits each. After estimating the baseline IOP at 9 AM, one of the randomly chosen eyes was instilled with 50 μl of DCE/pilocarpine, while the contralateral eye received the same volume of vehicle. Subsequently, IOP estimation was done at an interval of 1 hr until the baseline IOP was achieved.

The side effects of DCE/pilocarpine were noted including circumcilliary congestion, pupillary miosis and flare and cells in anterior chamber.

Results

IOP lowering effect of Daucus carota on normotensive rabbit eyes—Untreated normotensive

rabbits showed a mean % IOP change of -6.14 to 3.15% from baseline (Fig. 1a). Treatment with DCE (0.3%) resulted in mean peak IOP reduction of 19.33% from baseline at 4 hr post drug instillation. Mean peak IOP lowering of 23.20 and 25.61% from baseline was observed at 2.5 hr after instillation of DCE 0.6 and 1.2% respectively. The mean peak IOP reduction with DCE 0.3% was significantly lower than DCE 0.6% and 1.2%. No significant difference was observed between mean peak IOP reduction caused by DCE 0.6 and 1.2% (Fig. 1b). Therefore, DCE 0.6% was chosen for further evaluation of IOP lowering effects in water loading and steroid induced models.

IOP lowering effect of Daucus carota (0.6%) on water loaded rabbit eyes—The untreated water loaded rabbits showed a maximum % rise in IOP from 0.75-1.00 hr post water loading (Fig. 2a). Therefore, DCE was instilled 1.75 hr before water loading so that the peak effect of the test drug coincides with the peak rise in IOP. Similarly, pilocarpine was instilled 0.25 hr before water loading as it has shown peak effect at 1.00 hr in normotensive rabbits in preliminary experiments. In response to water loading the rabbits unilaterally instilled with DCE (0.6%) showed significantly lower rise in IOP of treated eye as compared to control eye. The treated eye showed a mean IOP rise of 37.92% from baseline 0.25 hr post water loading whereas, the same was 58.79% in the control eye. Significant difference in mean IOP rise in treated and control eyes persisted throughout the experimental period. Maximum mean difference of 29.39% between the treated and control eyes was observed at 0.75 hr post water loading (Table 1, Fig. 2b).

Pilocarpine treated group also showed a significant difference between the mean IOP rise in treated and control eyes and this was observed up to 1.50 hr post

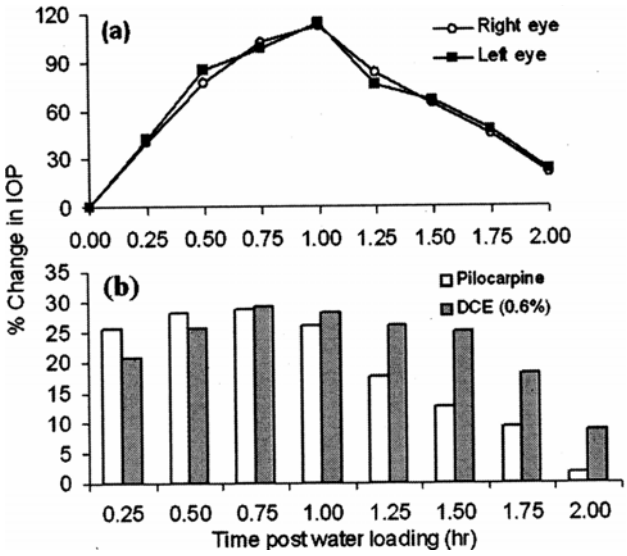

Fig. 2—(a) Water loading induced rise in IOP in normal untreated rabbits.(n=24 eyes). (b) Effect of pilocarpine and DCE (0.6%) on water loading induced oculohypertension in rabbits. [Each bar represents the mean difference between % rise of IOP in treated and control eyes, 0.25-2.00 hr post water loading.(n=12 eyes).]

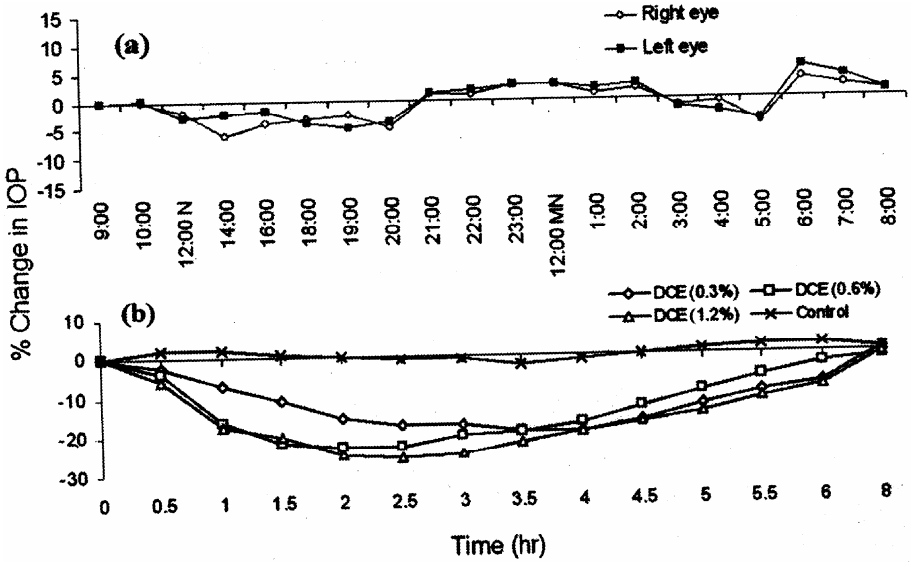

Fig. 1—(a) Diurnal variation of normal rabbit IOP (n=24 eyes). (b) Effect of three concentrations of *Daucus carota* aqueous extract on normotensive rabbit eyes. [Each data point represents (%) difference of IOP from baseline. As the (%) change in IOP in control eyes did not differ among the three dose groups single curve represents the control for all three doses.(n=12 eyes).]

water loading. Maximum mean % IOP difference of 28.91 between % IOP rise of test and control eyes was observed at 0.75 hr post water loading (Table 1, Fig. 2b).

IOP lowering effect of Daucus carota (0.6%) on steroid pretreated rabbit eyes - Bilateral instillation of prednisolone resulted in an IOP elevation of 31.58% from baseline at the end of 40 days and this change in IOP was significantly greater than that in saline treated group. Thereafter an elevated IOP was sustained for 4 weeks (Fig. 3a). A mortality of 20% was observed during the period of steroid instillation.

Unilateral instillation of DCE (0.6%) in steroid pretreated rabbit eyes showed maximum mean IOP reduction of 30.27% at 3 hr post drug instillation. Significant difference between mean IOP of treated and control eyes persisted from 1-6 hr post drug instillation. (Table 1, Fig. 3b).

Significant difference between mean IOP of treated and control eyes was observed from 1-6 hr in

Table 1—IOP lowering effects of pilocarpine and *Daucus carota* in water loading and steroid induced models.
[Values are mean ± SD of 12 eyes]

Water loading model					Steroid induced model				
Time (hr)	Pilocarpine		DCE (0.6%)		Time (hr)	Pilocarpine		DCE (0.6%)	
	TE	CE	TE	CE		TE	CE	TE	CE
0.00	7.28 ± 0.89	7.38 ± 0.85	6.45 ± 1.43	6.59 ± 1.36	0	7.18 ± 0.65	7.26 ± 0.59	8.46 ± 2.08	8.66 ± 2.13
0.25	9.37 ± 1.16*	11.39 ± 1.32	8.84 ± 2.03*	10.41 ± 2.09	1	5.32 ± 0.54*	7.09 ± 0.60	6.83 ± 1.73*	8.63 ± 2.01
0.50	11.04 ± 1.20*	13.26 ± 1.19	10.22 ± 2.30*	12.08 ± 2.28	2	5.63 ± 0.56*	7.13 ± 0.57	5.99 ± 1.52*	8.37 ± 2.01
0.75	12.46 ± 1.46*	14.72 ± 1.35	11.25 ± 1.88*	13.41 ± 2.04	3	5.90 ± 0.65*	7.16 ± 0.70	5.86 ± 1.39*	8.37 ± 1.87
1.00	12.28 ± 1.49*	14.33 ± 1.25	10.87 ± 2.40*	12.94 ± 2.49	4	6.24 ± 0.50*	7.11 ± 0.45	6.28 ± 1.32*	8.40 ± 1.83
1.25	10.73 ± 1.08**	12.24 ± 0.99	9.31 ± 1.30*	11.37 ± 1.93	5	6.65 ± 0.58***	7.11 ± 0.60	7.17 ± 1.77*	8.54 ± 1.97
1.50	9.81 ± 0.95**	10.90 ± 1.11	8.06 ± 1.26**	9.92 ± 1.56	6	6.89 ± 0.56***	7.22 ± 0.60	7.76 ± 1.89**	8.63 ± 1.96
1.75	8.32 ± 0.39	9.15 ± 0.79	7.13 ± 1.60**	8.44 ± 1.63	8	7.03 ± 0.56	7.22 ± 0.67	8.26 ± 1.92	8.62 ± 2.06
2.00	8.48 ± 0.80	8.72 ± 0.87	6.97 ± 1.22***	7.72 ± 1.50					

P values: significant at *<0.0001, ** <0.001, *** <0.01 as compared to corresponding control, TE= Treated eye, CE= Control eye

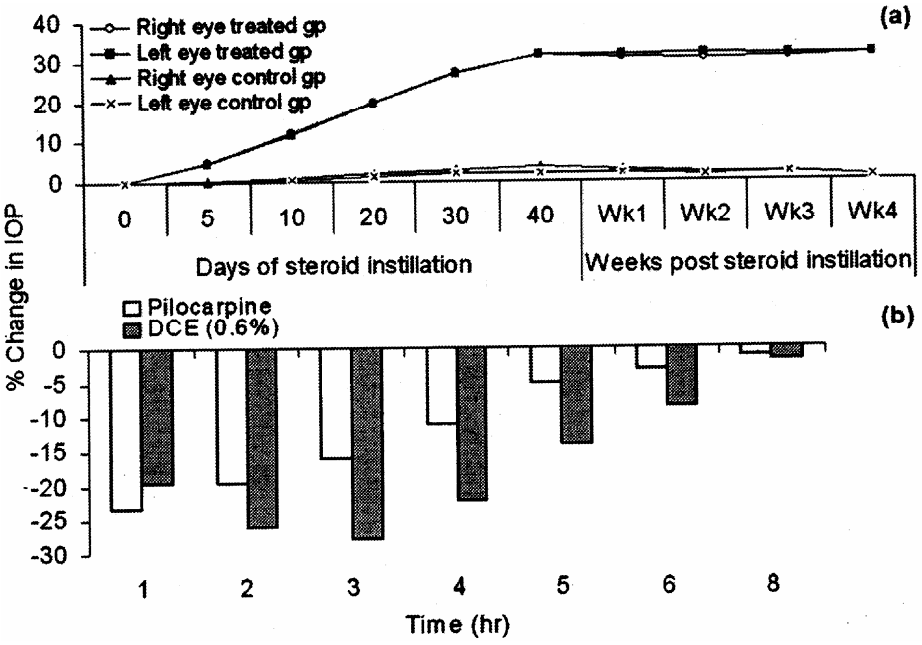

Fig. 3—(a) Steroid induced elevation of IOP (n=32 eyes) (b) Effect of pilocarpine and DCE (0.6%) on steroid induced oculohypertension in rabbits during 1st week post steroid. [Each bar represents the mean difference between the % rise of IOP in treated and control eyes, 1-8 hr post drug instillation. (n=12 eyes).

pilocarpine treated group. Maximum mean IOP reduction of 25.65% from baseline was observed at 1 hr post pilocarpine instillation. (Table 1, Fig. 3b).

Side effects—All rabbit eyes treated with pilocarpine showed pupillary constriction. No signs of ocular irritancy were observed in rabbits treated with DCE.

Discussion

Daucus carota is known for its beneficial effects on ocular health because of its high contents of beta-carotene. However, the effect of *Daucus carota* seed extract on intraocular pressure was studied for the first time. This study clearly demonstrated the IOP lowering effects of aqueous extract of *Daucus carota* seeds in rabbits with normal and experimentally elevated IOP.

In rabbits with normal IOP, instillation of a single drop of DCE resulted in significant IOP reduction. It was also observed that the peak IOP reduction with DCE 0.3% was achieved at 4 hr post drug instillation, while the same was at 2.5 hr with DCE 0.6% indicating a more rapid onset of action with increasing concentration. Besides, the peak IOP reduction with DCE 0.6% was significantly higher than DCE 0.3%. Further increase in concentration from 0.6 to 1.2% neither caused a similar reduction in the time for onset of action nor a significantly higher peak IOP reduction.

IOP lowering drugs are expected to protect against the water loading induced rise in IOP when instilled prior to intragastric administration of water. Both DCE (0.6%) and pilocarpine effectively protected against the acute rise in IOP when instilled 1.75 hr and 1 hr, respectively, before water loading. However, pilocarpine provided significantly higher protection against rise in IOP during first 0.5 hr while the efficacy of DCE was better during the 2nd hour post water loading. At 0.75 and 1.00 hr post water loading (the period of peak rise in IOP) the efficacy of DCE was equivalent to that of pilocarpine. These results indicated the slower onset of action of DCE (0.6%) as compared to pilocarpine however, the peak effect and the duration of action of DCE (0.6%) are comparable to that of pilocarpine.

In steroid model pilocarpine instillation resulted in significantly higher IOP reduction as compared to DCE (0.6%) at 1 hr post drug instillation. From 2-6 hr post drug instillation, the IOP reduction with DCE (0.6%) was significantly greater than pilocarpine. Moreover, the mean peak IOP reduction achieved with pilocarpine was significantly lower than DCE (0.6%). These results further confirmed the slower onset of action of DCE (0.6%) as compared to pilocarpine. Maximum IOP lowering with DCE was significantly greater than that with pilocarpine however, the total duration of action of two drugs was comparable.

A comparatively faster onset of action following pilocarpine instillation can be attributed to its mechanism of action. Pilocarpine, a cholinergic drug, reduces intraocular pressure by causing contraction of ciliary muscle thereby, exerting a pull on scleral spur and opening the aqueous drainage channels in trabecular meshwork. This mechanism of action was also evidenced by pupillary constriction observed in all rabbits instilled with pilocarpine and this effect is also well known in human. Further, a comparatively low peak IOP lowering effect of pilocarpine in steroid induced glaucoma model can also be attributed to its mechanism of action as the drug acts by improving aqueous drainage at pretrabecular sites, while in steroid induced glaucoma the site of obstruction to aqueous drainage lies within the trabecular meshwork. We chose to compare DCE with pilocarpine due to known cholinergic properties of the water-soluble fractions from alcoholic seed extract of *Daucus carota*[5,6]. However no pupillary constriction was observed in DCE treated eyes suggesting a relatively weak cholinergic activity. Further, the efficacy of cholinergic drugs like pilocarpine in steroid model is reported to be much lower than other IOP lowering drugs like timolol and latanoprost[11]. The observations in steroid model showing significantly higher IOP reduction with DCE as compared to pilocarpine are suggestive of involvement of additional mechanisms such as improved uveoscleral outflow in lowering IOP. Also the effect on ciliary processes to modulate the aqueous secretion cannot be ruled out. Further investigations to isolate the active constituents and study the mechanism of action will reveal the true IOP lowering potential of *Daucus carota.*

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References

1 Gilani A H, Shaheen E, Saeed S A, Bibi S, Irfanullah , Sadiq M & Faizi S, Hypotensive action of coumarin glycosides from *Daucus carota*, *Phytomedicine*, 7 (2000) 423.

2 Mahran G H, Kadry H A, Isaac Z G, Thabet C K, Al-azizi M M & El-Olemy M M, Investigations of diuretic drug plants, 1. Phytochemical screening and pharmacological evaluation of *Anethum graveolens* L., *Apium graveolens* L., *Daucus carota* L. and *Eruca sativa* Mill. *Phytotherap Res*, 5 (2006) 169.

3 Nicolle C, Cardinault N, Aprikian O, Busserolles J, Grolier P, Rock E, Demigné C, Mazur A, Scalbert A, Amouroux P & Rémésy C, Effect of carrot intake on cholesterol metabolism and on antioxidant status in cholesterol-fed rat, *Eur J Nutr*, 42 (2003) 254.

4 El S N & Karakaya S, Radical scavenging and iron-chelating activities of some greens used as traditional dishes in Mediterranean diet, *Int J Food Sci Nutr*, 55 (2004) 67.

5 Gambhir S S, Sanyal A K, Sen S P & Das P K, Studies on *Daucus carota* Linn. II. Cholinergic activity of the quaternary base isolated from water-soluble fraction of alcoholic extract of seeds, *Indian J Med Res*, 54 (1966) 1053.

6 Vasudevan M & Parle M, Pharmacological Evidence for the Potential of *Daucus carota* in the Management of Cognitive Dysfunctions, *Biol Pharm Bull*, 29 (2006) 1154.

7 Gupta S K, Saxena R, Agarwal R, Galpalli N, Srivastava S & Agrawal S S, Estimation of intraocular pressure using Non Contact Tonometer in rabbits: A comparative evaluation with Schiotz tonometer, *Meth Find Clin Exp Pharmacol*, 29 (2007) 405.

8 Thorpe R M & Kolker A E, A tonographic study of water loading in rabbits, *Arch Ophthalmol*, 77 (1967) 238.

9 McDonald T O, Hodges J W & Borgmann A R, The water-loading test in rabbits, *Arch Ophthalmol*, 82 (1969) 381.

10 Melena J, Santafe J & Segarra J, The effect of topical diltiazem on the intraocular pressure in betamethasone-induced ocular hypertensive rabbits, *Pharmacol Exp Therap*, 284 (1998) 278.

11 Gupta S K, Agarwal R, Galpalli N D, Srivastava S, Agrawal S S & Saxena R, Comparative efficacy of pilocarpine, timolol and latanoprost in experimental models of glaucoma. *Meth Find Exp Clin Pharmacol*, 29 (2007) 665.