

## IOP Lowering Effects of *Ocimum basilicum* Seed Extract in Two Rabbit Models of Ocular Hypertension

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### ABSTRACT

**Introduction:** *Ocimum basilicum* (OB), a herb known for its antihypertensive, anticholinesterase and antioxidant properties was investigated for possible intraocular pressure (IOP) lowering effects in rabbits with ocular hypertension (OHT). **Methods:** The IOP lowering effect of a single drop of OB extract (OBE) was evaluated in oculonormotensive rabbits using three concentrations (0.25, 0.5 and 1% w/v). The concentration showing maximum IOP reduction was further evaluated in rabbits with water-loading and steroid-induced OHT. **Results:** IOP lowering effect of OBE 0.5% in oculonormotensive rabbit eyes was significantly greater compared to OBE 0.25% ( $p < 0.05$ ) but was comparable ( $p > 0.05$ ) to OBE 1%. Therefore, 0.5% concentration was selected for further evaluation. Pretreatment with OBE (0.5%) caused significantly lower increase in IOP after water loading amounting to 23.39% above baseline as compared to 54.00% in control eye, 15 minutes post water loading. At 60 minutes, post water loading, mean IOP rise was 95.12% and 63.58% in control and test eyes, respectively. Significant difference between the mean IOP of two eyes persisted during the 2nd hr. In rabbits with steroid induced OHT, OBE 0.5% produced a mean IOP reduction of 24.73% at the end of first hr and the mean peak IOP reduction of 31.63% was observed at the end of 2 hr. A significant difference between the IOP of test and control eyes persisted from 1 to 6 hr. **Conclusions:** *Ocimum basilicum* seed extract showed significant IOP lowering effect in rabbits with water loading and steroid induced OHT, however, its utility as an effective antiglaucoma medication needs further investigations.

**KEYWORDS:** *Ocimum basilicum*, experimental glaucoma, water loading-induced, rabbits

### INTRODUCTION

Glaucoma, the leading cause of irreversible blindness worldwide, is characterized by apoptotic loss of retinal ganglion cells (RGCs). Elevated intraocular pressure (IOP) is one of the major but not the only risk factor in the pathogenesis of glaucoma. Despite the evidence showing involvement of several other factors such as increased oxidative stress in the pathophysiology of glaucoma [1], all currently available drugs primarily act by reducing IOP. However, it is widely known that a substantial number of patients show glaucomatous changes in the absence of elevated IOP and there are others who continue to have disease progression despite adequate control of IOP. Hence, the drugs with added properties, such as antioxidant effect, may be of

greater benefit in the prevention of RGC loss in glaucomatous eyes.

*Ocimum basilicum* L. is an annual and perennial herb and shrub, which is native to the tropical and warm temperate regions. It is also known as common basil or the sweet basil. Species of *Ocimum* have been used for centuries in Ayurvedic medicine. Among all *Ocimum* species, *Ocimum basilicum* is the most widely used species for medicinal purposes. Phytochemical screening of *Ocimum basilicum* showed the presence of glycosides, mucilages, gums, amino acids, proteins, phenolic compound, tannins, triterpenoids, saponins, sterols, and flavonoid [2]. The *Ocimum basilicum* seed extract was found to be rich in linalool, methyl cinnamate and



methyl chavicol. Seeds are also rich in unsaturated fatty acids including  $\alpha$ -linolenic, linoleic and oleic acids. Among the saturated fatty acids are palmitic and stearic acids [3]. A large number of therapeutic benefits of *Ocimum basilicum* can be attributed to its potent antioxidant activity [4]. Anticholinesterase activity of *Ocimum basilicum* has also been demonstrated [5], which may significantly contribute to its IOP lowering effect. Another study has demonstrated antihypertensive properties of *Ocimum basilicum* [6]. Keeping in view such wide-ranging properties of this medicinal plant we investigated IOP lowering effects of *Ocimum basilicum* in rabbits with experimentally-induced ocular hypertension. These effects were compared with those of timolol, a beta blocker. The relevant pharmacological activities predicting the mechanisms of action of *Ocimum basilicum* remain largely unknown, however, linalool, the major monoterpene from *Ocimum basilicum* is known to reduce blood pressure in spontaneously hypertensive rats [7]. Since, timolol, a currently used IOP lowering agent, belongs to the group of beta adrenergic blockers that reduce blood pressure, it is likely that there is similarity in their mechanisms of action. Hence, we chose timolol as the reference standard to compare the IOP lowering effects of *Ocimum basilicum*.

## METHODS

### Animals

Animal care and all the work done in these studies was in accordance with ARVO statement for the use of animals in ophthalmic and vision research. The study was approved by the Institutional Animal Ethics Committee. New Zealand white rabbits, weighing 2.00 - 2.5 Kg, were procured from a rabbit run at Delhi Institute of Pharmaceutical Sciences and Research and were subjected to systemic and ophthalmic examination. Those found normal were included in the study. Animals were individually housed and were maintained under standard laboratory conditions with room temperature at  $23 \pm 2^\circ\text{C}$  and photoperiod of 12D:12L. Pellet diet and tap water was accessible *ad libitum*.

### Plant Extract

Dried aqueous extract of the *Ocimum basilicum* seeds (OBE) was generously provided by Promed Exports Private Ltd, India. and was authenticated by HPTLC finger printing. The extract was stored at  $-20^\circ\text{C}$  until used. Eye drops were prepared fresh everyday by dissolving appropriate amount of extract in 0.25% HPMC depending on the required concentration. The solution was prepared in sterile vials and was filtered through 0.22  $\mu\text{m}$  Millipore filter to ensure that the solution was sterile. HPMC was used to increase the corneal residence time of the extract. Timolol 0.5% was used as reference standard.

### Experimental Model and Study Design

The IOP lowering effect of OBE was studied in rabbit eyes with normal and elevated IOP. Ocular hypertension in rabbits was achieved (1) by water loading (2) chronic steroid instillation. All IOP measurements were done by 2 masked observers and the average of the two independent readings was considered as the final estimate.

### The IOP Measurement

IOP was measured with a non-contact tonometer (Nidek-2000, Japan), which does not require local anesthesia. The technique of use of the noncontact tonometer in rabbits was as described previously [8].

### Effect of OBE on IOP in Ocular Normotensive Rabbits

The IOP of normal rabbits (2.00 – 2.50 Kg) was measured over a 24-hour period to assess the extent of normal diurnal variation. Thereafter, IOP lowering effect of OBE was studied at three dose levels: 0.25, 0.5 and 1% (w/v). Each dose group consisted of 12 rabbits. Animals were instilled with 50  $\mu\text{l}$  of extract solution in one of the randomly chosen eye after measuring the baseline IOP at 9.00AM. An equal volume of vehicle (0.25% HPMC) was instilled in the contralateral eye. Subsequently, IOP measurements were done at one-hour intervals until baseline IOP was achieved. Among the three doses, the one showing maximum % reduction in IOP from baseline was chosen for further evaluation in rabbits with experimentally elevated IOP.

### Effect of OBE on IOP in Ocular Hypertensive Rabbits

The concentration of OBE that showed maximum peak IOP reduction in normotensive model was chosen for further evaluation in rabbits with water loading and steroid-induced ocular hypertension.

### Rabbits with Water-loading Induced Ocular Hypertension

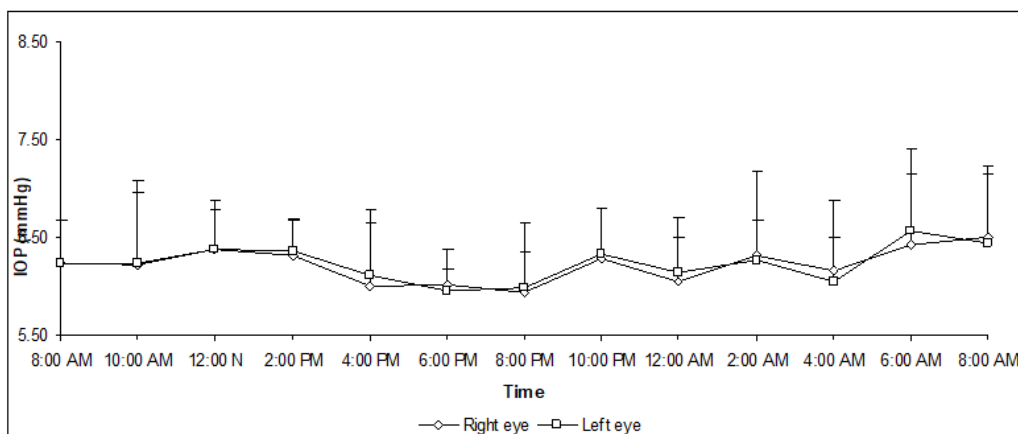
Experimental elevation of IOP was achieved by administration of tap water through an orogastric tube in conscious rabbits (2.00 – 2.5 Kg) using a volume of 70 ml/kg. Firstly, the time course of IOP rise was determined using six rabbits (n=12 eyes). After measurement of baseline IOP of both eyes, water was rapidly administered and IOP measurements were done at 15 min interval for a period of 120 min.

Subsequently, the effect of OBE on the IOP in oculohypertensive rabbit eyes was determined. Another group of 24 animals was randomly divided into timolol or the OBE treatment groups each consisting of 12 animals. The baseline IOP was measured and this was followed by instillation of 50  $\mu$ l of timolol or OBE unilaterally in one of the randomly chosen eyes and same volume of vehicle in the other eye. This was followed by water loading as described above. The time interval between the drug instillation and water loading was chosen so as the peak IOP

lowering effect of OBE (as determined in ocular normotensive rabbits) coincides with the time of maximum IOP rise in water loaded animals. After water loading, IOP measurements were done at 15 min interval for a total of 120 min.

### Rabbits with Steroid Induced Ocular Hypertension

The steroid-induced ocular hypertension was achieved in rabbits by topical instillation of steroids. Young rabbits (1.2-1.5 kg) were bilaterally instilled with 10  $\mu$ l of prednisolone acetate suspension 1.0% (Alcon, Inc.) twice daily for 40 days and IOP was measured on days 5, 10, 20, 30 and 40. Previous studies have shown that maximum IOP rise is obtained after 36-40 days of steroid instillation in rabbit eyes and no further increase in IOP occurs with continues steroid instillation [11-14]. Therefore, IOP lowering effect of OBE was studied on day 41 of prednisolone treatment. Twenty-four rabbits that achieved ocular hypertension by day 40 post steroid treatment, were divided into 2 groups of 12 rabbits each. All animals were subjected to baseline IOP measurement and this was followed by instillation of 50  $\mu$ l of the test drug (timolol or OBE) in one of the randomly chosen eye. The same volume of the vehicle was administered to other eye. Subsequently, IOP was measured every 60 min for a total of 8 hr.



**Figure 1** Diurnal variation in IOP of normal rabbits. All data points represent the mean  $\pm$  SD. n=12

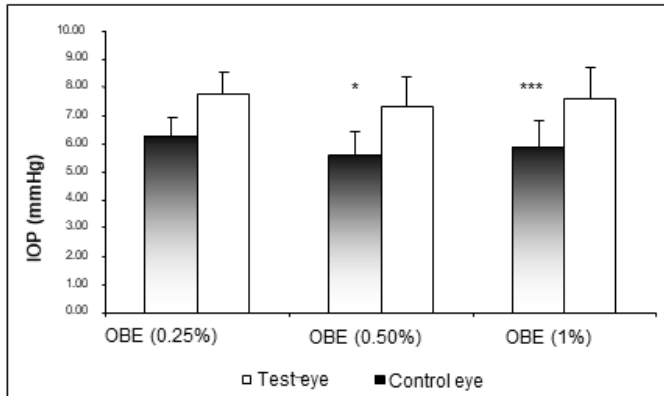
**Statistical Methods**

All data is expressed as mean ± SD. Unpaired t test was used for intergroup comparisons whereas paired t test was performed for within-group comparison. p<0.05 was considered statistically significant.

**RESULTS**

**Diurnal Variation**

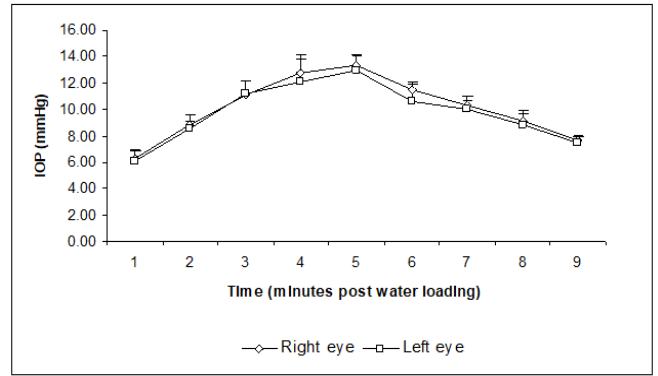
The IOP measurement over a period of 24 hr in both eyes of 6 rabbits (12 eyes) did not show significant changes during the period of observation. During the entire period of observation the mean IOP of both eyes remained close to baseline with maximum mean reduction of 4.89% and maximum elevation of 4.19% observed at 8 PM and 6.00 AM respectively. No significant difference was observed between the IOP of the two eyes at all time points (Figure 1).



**Figure 2** Peak IOP lowering effect of the single drop application of three concentrations of OBE, 3 hr post-instillation. Data presented as mean ± SD, n=12 \*p<0.05 versus OBE (0.25%), \*\*\*p<0.01 versus OBE (0.25%).

**Effect of OBE on Normal Intraocular Pressure**

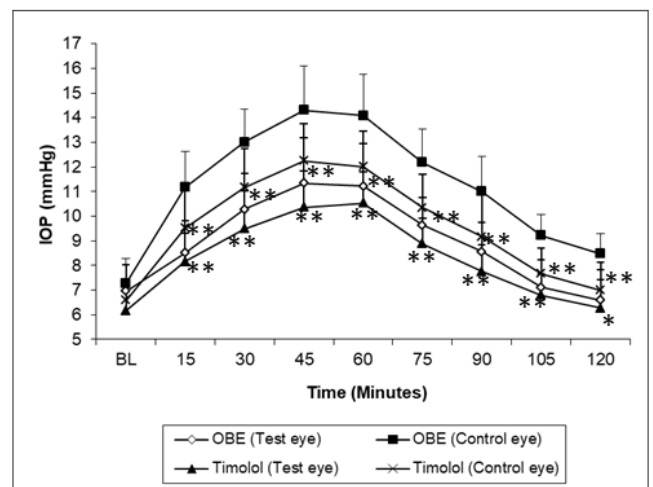
Unilateral instillation of OBE 0.25% to normotensive rabbit eye resulted in a maximum of 16.84% reduction from baseline. OBE, 0.5 and 1% resulted in a maximum mean IOP reduction of 22.66 and 23.10% respectively. The IOP reduction caused by OBE 0.5 and 1% did not differ significantly from each other (p>0.05) but a significant difference was observed between mean IOP reduction with OB 0.25% versus 0.5% (p<0.05) and OBE 0.25 versus 1% (p<0.01) (Figure 2). Keeping in view that the IOP lowering effect of OBE 0.5% and 1% was comparable; we used 0.5% concentration for further evaluation in rabbits with ocular hypertension.



**Figure 3** Rise in IOP of normal rabbits after water loading. All data points represent the mean ± SD, n=12

**Effect of OBE on Water-loaded Ocular Hypertensive Rabbit Eyes**

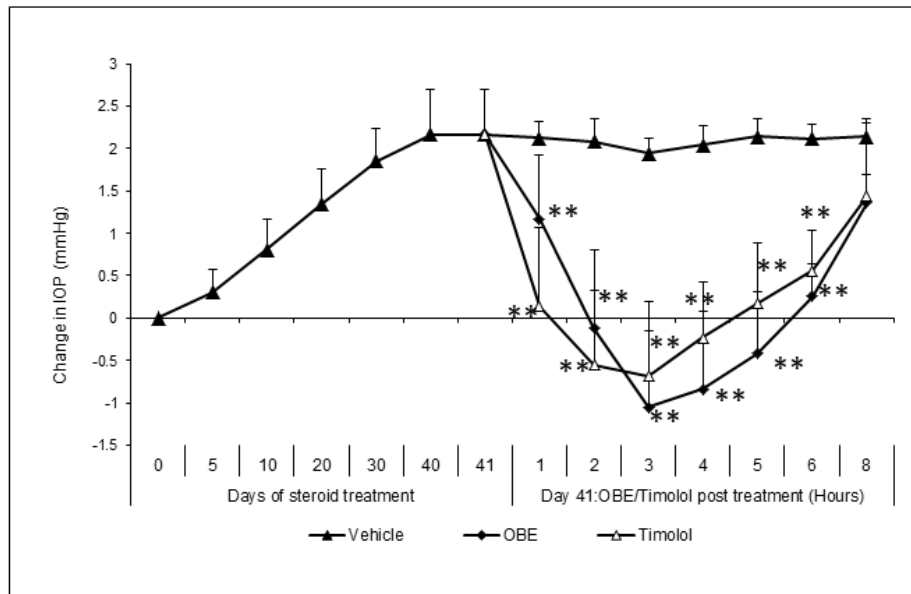
Time course of change in IOP in response to water loading over a period of 120 min showed a significant rise in IOP in both eyes of the 6 rabbits (12 eyes). Fifteen min post water loading, mean percent rise from baseline in right and left eyes was 40.08 and 42.34% respectively. At 30, 45- and 60-min post water loading the right eye showed a mean percent rise of 76.79, 102.66 and 112.65%, respectively from baseline. At the same time points the left eye showed a mean percent rise of 85.39, 98.54 and 114.76% respectively. No significant difference was observed between two eyes at any time point mentioned above (p>0.05). During the second hour also, there was a significant rise in IOP of both eyes but no significant difference was observed between the two eyes at all time points (Figure 3).



**Figure 4** Effect of OBE on IOP in water loaded ocularhypertensive rabbits. All data points represent the mean ± SD, n=12. \*p<0.05 versus corresponding control at the same time point; \*\*p<0.001 versus corresponding control at the same time point

Unilateral instillation of OBE 0.5% in water loaded rabbits resulted in a significantly lower rise in IOP at all time points during the period of observation after water loading. Fifteen min post water loading the mean rise in IOP of was 54.00% and 23.39% in control and test eye, respectively. At the end of 1st hr post water loading the mean rise in IOP was 95.12% and 63.58% above baseline in control and test eye,

respectively. The peak effect of OBE was observed at 45 min post water loading. The significant difference between the mean IOP rise of two eyes persisted during second hr with control eye showing 68.82, 52.46, 27.56 and 17.41% mean rise and test eye showing 40.79, 24.66, 10.69 and 4.04% mean rise above baseline at 75, 90, 105- and 120-min post water loading, respectively ( $p < 0.001$ ). (Figure 4).



**Figure 5** Effect of OBE on IOP in steroid-induced ocular hypertensive rabbits. All data points represent the mean  $\pm$  SD.  $n=12$ . \*\* $p < 0.001$  versus corresponding control at the same time point.

### Effect of OBE on Steroid-treated Ocular Hypertensive Rabbit Eyes

At the end of the 40 days of prednisolone treatment, an IOP rise of 31.58% was observed along with a mortality of 20%. After instillation of OBE, the mean IOP reduction of 24.73% was observed at the end of the first hour and the mean peak IOP reduction of 31.63% was observed at the end of the second hour. A significant difference between the IOP of test and control eyes persisted from 1 to 6 hr. At 8 hr post instillation, the IOP of test and control eyes was comparable. (Figure 5).

### DISCUSSION

Present study for the 1st time has demonstrated IOP lowering effect on OBE in rabbit models of water loading and steroid induced ocular hypertension. Among the three concentrations of OBE used, 0.5% showed significantly higher IOP reduction as compared to 0.25% in normotensive rabbit eyes.

However, this IOP reduction was comparable to that caused by 1% concentration. Therefore, 0.5% was chosen for further evaluation in rabbits with water-loading and steroid-induced ocular hypertension.

The method of elevating IOP in rabbits via intra-gastric water load has been utilized previously for evaluation of the effects of drugs in experiments of short duration employing anesthetized rabbits only [9, 10]. The use of this method has also been described earlier in conscious rabbits [11-13] as was the case in the current study. The water loading induced model of ocular hypertension in rabbits shows some desirable characteristics such as (1) neither the aqueous fluid production nor the outflow components of the eye are damaged, leaving these sites available for drug action; (2) there is no eye irritation to affect baseline IOP or pupil size; (3) IOP elevation is reproducible (4) similar responses are reliably obtained in both eyes of the same animal; IOP elevation by this method has been shown to be susceptible to inhibition by commonly

used anti-glaucoma drugs [14]. Furthermore, the elevated IOP induced in human glaucomatous patients as a result of the diagnostic "water-drinking test" [15, 16] suggests a possible analogy between glaucomatous human eyes and normal rabbit eyes in response to water load. It should be noted, however, that the animal model as presented measures prophylactic drug action rather than the ability to lower a sustained, elevated IOP as it exists in glaucomatous eyes. In the current study, the maximum efficacy of OBE (0.5%) in preventing the rise in IOP was comparable to timolol. The results also show that the time to onset of action of OBE, peak effect and duration of action are comparable to timolol ( $p > 0.05$ ).

We also studied the IOP lowering effect of OBE in rabbits with steroid-induced ocular hypertension. This method of experimental increase in IOP has been described in rabbits [11-13] and rats [17, 18]. Studies have shown that chronic topical application of steroid results in increased deposition of extracellular matrix components in the trabecular meshwork of eye, hence retarding the aqueous outflow and increasing the IOP [18-20]. Hence, steroid-induced model of ocular hypertension has been regarded as a useful method to study IOP lowering effect of experimental drugs [21]. In the current study, single drop of OBE caused a reduction in IOP by 31.63% from baseline. While the effect of OBE in hypertensive eyes of water-loaded rabbits indicated its usefulness for prophylaxis, its effect in steroid treated eyes indicates potential benefits in the treatment of ocular hypertension.

The mechanisms of IOP lowering effect of OBE have not been investigated so far. However, keeping in view the results of other studies it can be postulated that IOP lowering effect of OBE results from its action by multiple mechanisms modulating the aqueous humor dynamics. Linalool, a terpenoid from OB seeds, along with its other components has been shown to act on the cholinergic system of insects [22]. Jukic et al. demonstrated the anticholinesterase activity of monoterpenoids such as linalool [23]. Hence it is likely that OBE reduces IOP by inhibiting cholinesterase activity. Additionally, linalool is known to cause direct vasorelaxation [24, 25]. Since trabecular meshwork cells are known to possess

smooth muscle cells like properties [26], it is likely that OBE causes relaxation of trabecular meshwork resulting in enhanced aqueous humor drainage and consequently reduced IOP. Moreover, the antihypertensive effect of OBE has been attributed to its ability to prevent endothelin-1 activation. Such property of OBE may be of extreme benefit to patients with normal tension glaucoma, where the pathogenesis primarily involves vascular dysregulation. As opposed to currently available therapies, potent antioxidant properties of OBE indicate its potential as a neuroprotectant in the prevention of glaucomatous neuropathy. However, some of limitations of the current study must be considered. In the current study non-contact tonometer was used to measure IOP as described previously [8]. Although use of non-contact tonometer eliminated the effect of topical anaesthesia on IOP measurements, its use in clinical settings is now limited, hence to what extent the results could be extrapolated to humans needs further investigations. Further studies to isolate the active constituent(s) in OBE, their sites of action and effects on aqueous humor dynamics are of critical importance. Besides, the effects of OBE in modulating intraocular blood flow and oxidative stress need further evaluation to demonstrate its possible utility in the medical management of glaucoma.

### Conflicts of Interest

Authors declare none.

### Acknowledgement

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