



INTRAOcular PRESSURE LOWERING ACTIVITY OF OCUFORS[®] (FORSKOLIN 0.15% W/V OPHTHALMIC SOLUTION) IN WATER LOADED NEW ZEALAND WHITE RABBITS

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ABSTRACT

Intra Ocular Pressure (IOP) is the fluid pressure inside the eye and is the most important risk factor for glaucoma. From the roots of *Coleus forskohlii*, an aromatic herb growing all over India, we extracted Forskolin. In this study we studied the IOP lowering activity of two strengths of Ocufores, viz., Ocufores[®] (Forskolin 1% w/v ophthalmic solution) and Ocufores[®] (Forskolin 0.15% w/v ophthalmic solution) in 12 New Zealand white rabbits, divided into three groups, four rabbits each. IOP was induced using water loading model in all three groups, as an acute model, with the right eye of all the animals received water for injection and thereby served as reference control. Whereas the left eye on all the three group animals received active drug, with Group 1 animals instilled with one drop of 1% w/v Forskolin solution while Group 2 and 3 animals instilled with one and two drops of 0.15% w/v Forskolin solution respectively. Post instillation of Forskolin 1% w/v ophthalmic solution (one drop), Forskolin 0.15% w/v ophthalmic solution, (one drop) and Forskolin 0.15% w/v ophthalmic solution (two drops) showed an IOP reduction of 23%, 19% and 30% at 45min, 45min and 75min respectively in New Zealand white rabbits, under test conditions employed. Forskolin aqueous clear ophthalmic solution showed a prolonged reducing effect on the IOP with one drop of 1% (w/v). However, a similar effect was noticed with two drops of 0.15% (w/v) Forskolin aqueous ophthalmic solution. Further clinical evaluation and confirmation of the low strength Ocufores (Forskolin 0.15% w/v) ophthalmic solution would help in replacing its higher strength counterpart (Forskolin 1% w/v) in mild open angle glaucoma patients.

KEY WORDS: Intra Ocular Pressure, *Coleus forskohlii*, Forskolin, Ocufores, Glaucoma



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INTRODUCTION

Throughout the world, there are many drugs available for lowering IOP, acting through various mechanisms, either alone or as a combination. However, there is still paucity for effective medicines in the management of IOP with minimal side effects, and hence there is a substantial need for research of new drugs either synthetic or of natural origin that could address glaucoma successfully. Forskolin, a labdane diterpene (17- β -acetoxy-8, 13-epoxy-1 α , 6- β , 9 α -trihydroxylabd-14-en-11-one) obtained from the roots of *Coleus forskohlii* has been extensively studied by many researchers since 1980. However, the good effects of Forskolin could not be established through human studies since long time nor it was exploited commercially. Several investigators have studied the effects of Forskolin in the eye when applied topically; in 1983 Caprioli and Sears first reported that Forskolin suspension lowers the IOP in rabbit, monkey and human eyes by reducing the net aqueous inflow¹ which was confirmed by several reports²⁻⁸. Sami Labs Limited, a Bangalore based herbal and nutraceutical major in India, has worked extensively on this molecule. Following pre-clinical and two successful major clinical studies with 1% w/v Forskolin clear aqueous solution, the molecule at strength of 1% w/v has been approved by the Indian Drug Regulatory Authority in 2006 for the management of IOP and open angle glaucoma, and was branded as Ocufores[®]. A low strength of the same formulation, 0.15% w/v clear solution developed by Sami, was hypothesized to have a similar therapeutic effect like 1% w/v, irrespective of its strength. The aim of the current study was to establish pre-clinically that despite the low strength of 0.15% w/v aqueous clear solution, Forskolin is equivalent in its efficacy with that of its higher strength (1% w/v) counterpart.

Animals

The study was conducted in the animal research wing of Bioneds Private Limited, Bangalore with the protocol approved from sponsor, Sami Labs Limited. Twelve in-house bred New Zealand White male and female rabbits, aged 2-3 months, were used in the present study. The animals were housed individually under standard laboratory

conditions in an environmentally monitored, air-conditioned room with adequate fresh air supply (12-15 air changes per hour), room temperature 19.7- 22.9°C and relative humidity 53-67%, with 12 hours fluorescent light and 12 hours dark cycle. The temperature and relative humidity were recorded once daily. Nutrilab rabbit feed (manufactured by Provimi Animal Nutrition Pvt. Ltd.) was provided along with potable water *ad libitum* throughout the acclimatization (five days) and experimental period.

Preparation of Ocufores[®]

Forskolin 1.0 % w/v Ophthalmic Solution

Cavasol[®] W7 M PHARMA (obtained from Wacker Chemie AG, Germany) 200 g was dissolved in 1 liter of water for injection. With the formation of a clear solution, Forskolin (11.22 g) was added to the above solution and stirred for 24 hours in a closed vessel. Benzalkonium chloride (0.2 g) was added to the above solution and stirred. Disodium EDTA (1.0 g) was dissolved in a small quantity of water for injection and added to the above solution. The pH was adjusted to 5.5 - 6.0 with sodium hydroxide solution (0.1M). For Iso-osmolality, sodium chloride (3.0 g) was added in water for injection to the freshly prepared above solution. The final 1 liter solution was sterilized by filtering through 0.22 micron hydrophilic nylon filter under nitrogen atmosphere. The clear aqueous solution was analyzed for forskolin content by HPLC.

Forskolin 0.15% w/v Ophthalmic Solution

Forskolin (0.168g) was treated with a solution of 3 g of a derivative of β -cyclodextrin (namely randomly methylated β -cyclodextrin, CAVASOL[®]) in 100 ml of sterile water. The insoluble was filtered and removed. The clear solution was treated with antimicrobial preservatives recommended for ophthalmic solution. pH was adjusted to 5.5 -6.0 using 0.1M sodium hydroxide solution and the osmolality of 310 to 350 mOsmal / Kg was adjusted using sodium chloride. The preservatives used were benzalkonium chloride solution and Edetate disodium solution. The entire preparation was done in a strictly aseptic condition. The clear aqueous

solution was analyzed for forskolin content by HPLC.

Study Design & Drugs

On the last day of acclimatization, animals (6 males and 6 females) were randomly allotted to groups G1, G2 and G3 (2 males and 2 females per group). Clear colorless aqueous solutions of 1% w/v and 0.15% w/v developed by Sami Labs Limited were used as materials under test.

Rationale for using low strength

Earlier researchers had used 1 to 4 % w/v suspension of Forskolin in water for their Phase I and Phase II trials⁹. In these studies, a highly significant maximum reduction of IOP was 31 % and 25% respectively, reached at 2 and 6 hours after instillation of the forskolin drops. However, the amount of dissolved Forskolin in the suspension was only 0.035% as determined by HPLC analysis of the filtered solution. If 0.035% solution can produce a significant IOP reduction comparable to Timolol in those two human studies, we hypothesized that the dose in 1% Forskolin solution (DCGI approved product), earlier developed by us, could be reduced further. Accordingly, we developed 0.15% diluted solution (using sterile water) which was equivalent to 4% suspension that contained 0.14% of Forskolin in dissolved state developed and studied by earlier researchers (Meyer BH et al). This clear aqueous solution might help in the direct availability of dissolved Forskolin prepared and may address the inconvenience caused by glaucoma patients with such suspension developed by earlier researchers (Meyer BH et al). Besides this, the insoluble when filtered and removed may also have a drug effect as prominent on the intended application.

PROCEDURE

The study was performed in accordance with the recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animal facility published in the gazette of India, December 15th 1998 and also in accordance with the protocol approved by Institutional Animal Ethics Committee of Bioneds Private Limited (BIO- IAEC 1095). The experimental increase in IOP was

achieved using water loading model where IOP elevation was achieved in unanaesthetized rabbits according to the previously described procedure^{10, 11}. Conscious rabbits were rapidly administered with 70 ml/kg of tap water through an orogastric tube. On the day of experiment, basal IOP was measured using a tonometer (HS Climent Clarke International, MK2 model) and recorded for all the animals of Groups G1, G2 and G3. After basal IOP measurement in mm Hg, experimentally induced elevated IOP was achieved immediately after water loading that lasted for atleast 2 hours. Left and right eye of animals of G1, G2 and G3 groups were measured for IOP at 15, 30, 45, 60, 75, 90, 105 and 120 minutes post instillation of test items and sterile water for injection respectively in the conjunctival sac. Dose of one drop (approximately 50 μ L) of Ocufores[®] (Forskolin 1% w/v solution) was instilled in G1 group animal and one and two drops of Ocufores[®] (Forskolin 0.15% w/v solution) were instilled into the conjunctival sac of G2 and G3 animals' left eye respectively. Right eyes were instilled with an equivalent amount of sterile water and hence served as reference control.

RESULTS

Post instillation of single drop of Ocufores[®] (Forskolin 1% w/v ophthalmic solution) in to the left eye of each animal in Group 1 showed a significant difference in mean IOP at 30 minutes, 45 minute, 60 minutes, 75 minute, 105 minutes and a similar effect was noticed within 15 minutes and up to 60 minutes post instillation of a single drop of Ocufores[®] (Forskolin 0.15% ophthalmic solution) in Group 2. Post installation of two drops of Ocufores[®] (Forskolin 0.15% w/v ophthalmic solution) in to the left eye of each animal in Group 3 showed significant difference ($p < 0.05$) in mean IOP at 60, 75 and up to 105 minutes when compared to their respective right eyes (Graphs 1, 2 and 3). Even though the reduction in IOP was observed at 30 and 45 minutes with two drops of Ocufores, the results did not reach statistical significance. Student's 't' test was employed for comparing the difference in IOP between right and left eyes at various time intervals and a p- value less than 0.05 was considered statistically significant. Post instillation of one drop of

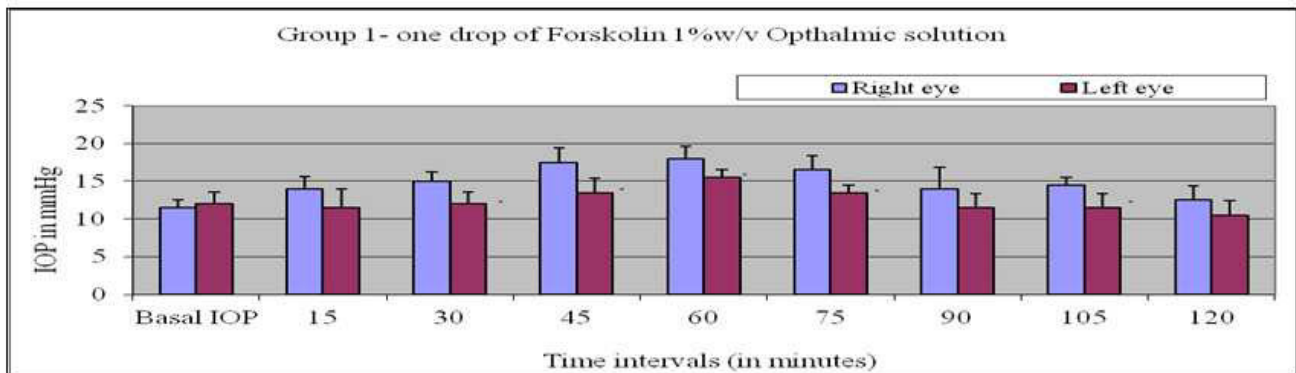
Ocufors[®] (Forskolin 1% w/v solution), one drop of Ocufors[®] (Forskolin 0.15% w/v solution) and two drops of Ocufors[®] (Forskolin 0.15% w/v solution) in the left eye of all the three group animals, showed maximum percent change (reduction) in IOP at 45 minutes, 45 minutes and 75 minutes with 23%, 19% and 30% respectively when compared to the right eye treated with equivalent amounts of sterile water (Graph 4).

Tolerability and Safety

Ophthalmological examination by indirect method was performed once during the

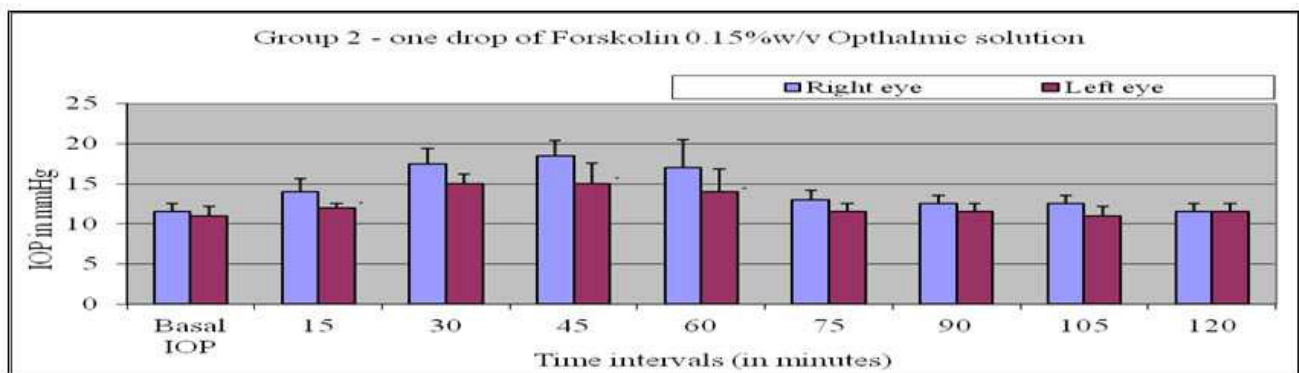
acclimatization period, and at the end of the study. At the end of the experimental period, there were no treatment related signs observed in all the animals. No abnormalities in eye adnexa, optic disc, tapetum lucidum, tapetum nigrum detected in all the groups of animals and hence it confirms that there is no toxicity associated with the formulations (1% and 0.15% w/v clear solutions) under study. The product at various strengths was found to be irritant free, safe with no reports of ocular toxicity (Table 1).

Graph1
EFFECT OF ONE DROP OF OCUFORS® (FORSKOLIN 1% W/V OPHTHALMIC SOLUTION)
ON THE INTRA OCULAR PRESSURE OF THE EYE



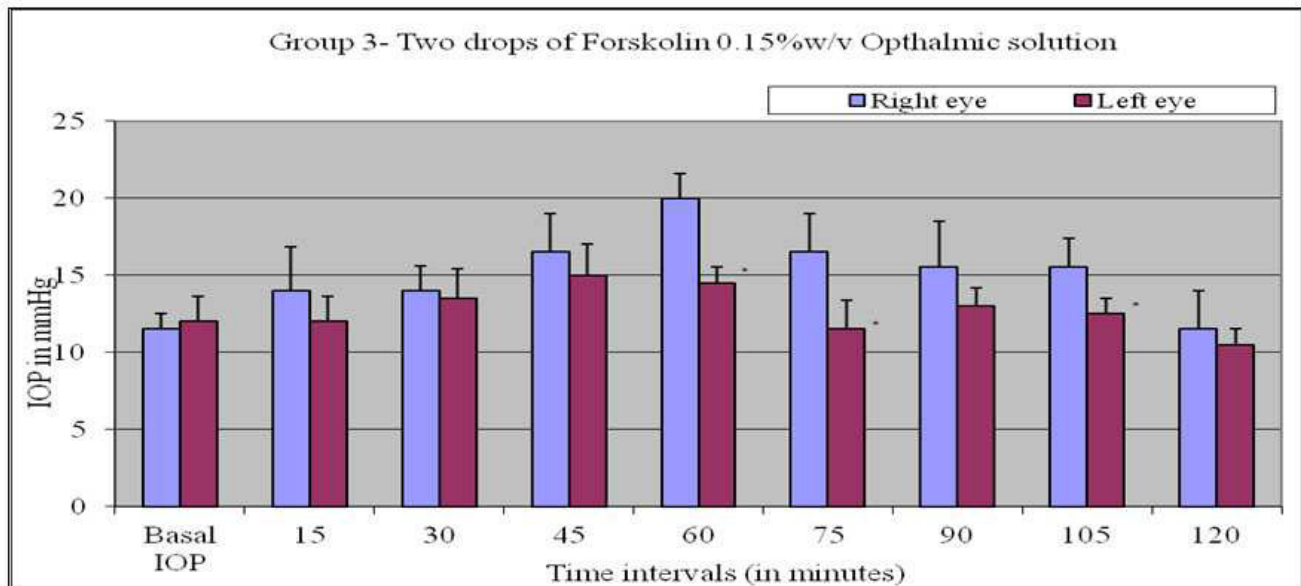
Right eye (Sterile water), Left Eye (Drug); difference in IOP was significant
* $p < 0.05$ at 30, 45, 60, 75 and 105 minutes post instillation of drug.

Graph2
EFFECT OF ONE DROP OF OCUFORS® (FORSKOLIN 0.15% W/V OPHTHALMIC SOLUTION)
ON THE INTRA OCULAR PRESSURE OF THE EYE



Right eye (Sterile water), Left Eye (Drug); difference in IOP was significant
* $p < 0.05$ at 15, 45 and 60 minutes post instillation of drug

Graph3
EFFECT OF TWO DROPS OF OCUFORS® (FORSKOLIN 0.15% W/V OPHTHALMIC SOLUTION) ON THE INTRA OCULAR PRESSURE OF THE EYE



Right eye (Sterile water), Left Eye (Drug); difference in IOP was significant * $p < 0.05$ at 60, 75 and at 105 minutes post instillation of drug

Graph4
PERCENTAGE CHANGE IN INTRA OCULAR PRESSURE

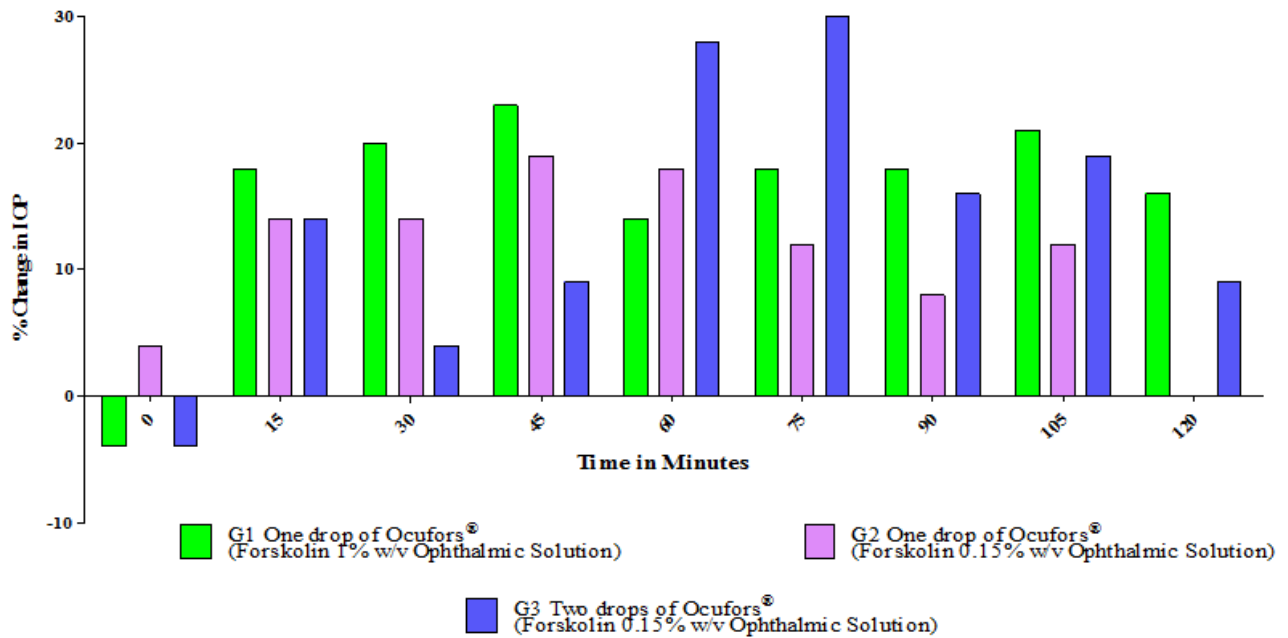


TABLE 1
INDIVIDUAL ANIMAL OPHTHALMOSCOPIC EXAMINATION

Group, Dose & Sex	G1 One drop of Ocufores® (Forskolin 1% w/v Ophthalmic Solution) & M,F								G2 One drop of Ocufores® (Forskolin 0.15% w/v Ophthalmic Solution) & M,F								G3 Two drops of Ocufores® (Forskolin 0.15% w/v Ophthalmic Solution) & M,F									
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8	9	10	11	12		
Animal No.	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
Adnexa	Lids	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	Ducts	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
	Cornea	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	
Iris	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		
Aqueous Humour	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		
Lens	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		
Vitreous Humour	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		
Retina	Vessels	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		
	Macula	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		
Optic disc	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		
Tapetum lucidum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		
Tapetum nigrum	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N		

N: Normal, A: Absent (Normal), M: Male, F: Female; L: Left, R: Right.
Instillation of mydriatic agent in both the eyes was carried out before ophthalmological examination.

DISCUSSION

Forskolin (17- β -acetoxy-8, 13-epoxy-1 α , 6- β , 9 α -trihydroxylabd-14-en-11-one), a labdane diterpene with the ability to activate cyclic AMP (cAMP) generation in a variety of eukaryotic cells (Seamon and Daly, 1981), is present in ethanolic extract from the roots of the Indian plant *Coleus forskohlii*. Forskolin activates adenylate cyclase in rabbit and freshly dissected human ciliary processes, as well as in human cultured ciliary epithelial cells. Timolol did not inhibit this activation, substantiating the finding that Forskolin does not work by interaction with the beta adrenergic receptor (Caprioli et al 1984). Forskolin, by elevating cAMP, is responsible for the pharmacological action of *Coleus forskohlii* root¹². In mammals, Forskolin is a positive inotropic and peripheral vasodilating agent (Lindner, Dohadwalla and Bhattacharya, 1978). Forskolin activates adenylate cyclase in membrane preparations and in whole cells from brain, kidney, thyroid, and other tissues, resulting in elevated cAMP levels similar to and sometimes greater than those resulting from stimulation of P-receptors or vasopressin (Cuthbert and Spayne, 1982; Fradkin, Cook, Kilhoffer and Wolff, 1982; Seamon and Daly, 1981; Caprioli et al., 1984). A pilot double-blind, placebo-controlled trial suggest that eye drops containing Forskolin, reduces intraocular pressure in people even without glaucoma¹³. For convenience of administration, pharmaceutical solution has to contain a therapeutic dose of the drug in required volume¹⁴. Earlier Meyer BH et al, in India had conducted two clinical trials on their Forskolin suspension. In those two studies, it was revealed that 1 to 4% w/v Forskolin suspensions showed similar IOP reduction without showing any concentration effect. A recent Chinese report has highlighted the significant IOP reducing property of Iso-forskolin (an isomer of Forskolin) at 0.05% concentration level suggesting that higher concentrations may not be required¹⁵. In view of these interesting reports, we filtered 4% w/v of Forskolin suspension used by earlier

researchers and developed a clear solution that contained 0.14% of Forskolin in a dissolved state. The experimental increase in IOP was achieved by water loading model with an acute rise in IOP in this animal study, which closely resembles the clinical situation with rapid rise in IOP. In the current confirmatory animal study, the safety and efficacy of 0.15% w/v Forskolin solution was assessed by comparing it with our DCGI (Drugs Controller General of India) approved 1% w/v Forskolin solution.

CONCLUSION

Our present study on evaluating the therapeutic equivalence of 1% w/v and 0.15% w/v of Forskolin clear solutions in reducing the IOP showed encouraging results. Interestingly, two drops (100 μ L) of 0.15% w/v of Forskolin had not only shown therapeutic equivalence at certain time intervals but also showed a larger percentage (%) of IOP reduction comparable to the standard 1% w/v one drop Forskolin solution at 60 min, 75 min up to 105 min post instillation in the New Zealand Rabbit Eyes, indicating it's sustained effect in the reduction of IOP. From our study it is evident that, one drop of 1% w/v solution of Ocufores[®] which shows a prolonged reducing effect on the IOP starting within 30 min and up to 105 minutes post instillation in the New Zealand Rabbit Eyes is comparable and a similar effect can be achieved using two drops of a lower strength of 0.15% w/v solution of Ocufores[®]. With no toxicity reported at any of the strengths and with its proven efficacy, clinical studies on evaluation of this newly developed low strength (0.15% w/v) Forskolin solution would help us to further understand and confirm its therapeutic activity in the management of IOP and open angle glaucoma. Lack of relatively large sample size and a standard drug receiving group are the limitations of this current study.

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