



THE EFFECT OF FORSKOLIN OPHTHALMIC INSERTS ON INTRAOCULAR PRESSURE IN RABBIT EYES.

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ABSTRACT

Forskolin, a diterpene obtained from natural roots of *Coleus forskohlii* (wild) Briq. (family: *Lamiaceae*), reduces Intra Ocular Pressure by 23-28%, which is a desirable feature for an antiglaucoma therapy. Ophthalmic Inserts of forskolin extract (OIE) and pure forskolin (OIF) were prepared as matrix controlled delivery with the aim of achieving once a day administration. Ocular safety studies by draize test, pharmacodynamic efficacy by schiøtz tonometry and precorneal residence by HPTLC technique were conducted in 30 New Zealand albino rabbits to investigate their safety for ocular administration. Obtained results concluded, that the Ophthalmic Insert Drug Delivery System (OIDDS) for forskolin shown a significant reduction in Intraocular Pressure up to 24 hours and an increased corneal residence time up to 12 hours with sustained therapeutic action which is a desirable feature for an ideal antiglaucoma agent.

Keywords: Forskolin; Ophthalmic inserts; Glaucoma; Intra ocular pressure.

INTRODUCTION

“VISION 2020, THE RIGHT TO SIGHT”, was the global initiative launched in the year 1999. It is estimated that, world-wide approximately 180 million people are visually impaired; of these between 40 to 45 million are blind. Even more compelling, it is estimated that the number of blind and visually impaired double by 2020, unless concerted action is undertaken to stem this toll. Glaucoma is the third leading cause of blindness worldwide and is responsible for about 52 million cases of blindness¹ (12.5%, based on the number of legally blind persons in the population at a given time). Glaucoma is an ocular disease caused by a progressive form of optic nerve damage associated with raised (>21mm of Hg) intraocular tension². It is a progressive optic neuropathy characterised by functional and structural impairment of three different ocular tissues – the Trabecular Meshwork (TM), Optic Nerve Head (ONH)

and Retinal Ganglionic Cells (RGCs) - which leads to deterioration of vision (loss of visual field) and, ultimately, blindness. Structural changes in the TM and ONH lead to elevated Intra Ocular Pressure (IOP) and to optic-disc cupping, respectively, and RGCs undergo progressive apoptotic cell death^{1,3}.

In this study, forskolin as a drug of choice is a diterpene obtained from natural roots of *Coleus forskohlii* (family: *Lamiaceae*). Forskolin has intra oculo-hypotensive effect^[11] and hence it is useful in the treatment of glaucoma. Advantages of forskolin in the treatment of glaucoma as an antiglaucoma agent has been reported for- reduction in IOP by 23-28% (instilled as 1% solution)⁴⁻⁸. It does not induce miosis– parasympathomimetics induce miosis⁹, which is not a desired effect in the treatment of glaucoma, increases intraocular blood flow– a desirable feature for antiglaucoma agent^{5,10}. The effect of forskolin can be increased and possibly potentiated by

the use of sympathomimetics, unilateral reduction of IOP. Advantage when only one eye is too treated. Less contraindication when compared to systemically active drugs.

In past several years, forskolin a diterpene derivative which directly activates the catalytic subunit of adenylate cyclase¹² has attracted attention as a potent antiglaucoma medicament. 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 inflow,¹³ which was confirmed by several reports¹⁴⁻²⁰. On the other hand Brubaker argued against forskolin's IOP lowering effect in human eyes. As forskolin has a low solubility in water, a part of this discrepancy in the IOP lowering effect might be explained by its poor ocular penetration. According to Brubaker²⁰ the significant decrease in IOP after topical application of 1% forskolin, Burstein¹⁵ documented this could be attributed to the fact that the performed tonography just before the application of the drug which could have increased corneal permeability and enhance the penetration of forskolin. The knowledge of herbal pharmacokinetics of topically applied drugs is essential for understanding its therapeutic effects²¹. Two studies were performed to investigate the effects of forskolin (Hoechst Research) on IOP, in first study two 1% formulations of eye drops were compared with placebo in 10 healthy volunteers, in a subsequent study only one formulation of 1% forskolin was compared with placebo by Meyer B. H⁷.

So, the extensive research is needed as per formulation aspects of ocular drug delivery concern of forskolin, which should be safer to eye. Ocular bioavailability of drugs from eye drops is poor due to precorneal loss factors including tear dynamics non productive absorption transient residence time in cul-de-sac and the relative impermeability of the cornea epithelial membrane. Only a small fraction of topically applied dose reaches the inner eye, with the actual amount dependent on the physicochemical properties of the drug and its vehicle. Glaucoma treatment needs the drug residence for longer duration in eye to control IOP, so that the pressure exerted on optic nerve will be less. As a result optic nerve damage will be prevented. Polymeric inserts increase the precorneal residence time for water insoluble drug. To overcome the limitations of using eye drops, the Ophthalmic Insert Drug Delivery System (OIDDS) will be superior to deliver the fraction of drug for longer duration with increased corneal residence time and bioavailability of drug. In this experiment, we have focused on formulation of ophthalmic inserts of *Coleus forskohlii* extract and forskolin designed to deliver forskolin in a sustained manner to maintain reduced IOP, and increased corneal residence time of forskolin, which was investigated in rabbit's induced glaucomatous eyes.

MATERIALS AND METHODS

Materials

Forskolin and its marker compound were obtained from Sami Labs Ltd., Bangalore, India. Sodium alginate, Polyvinyl alcohol-14000 (PVA), Hydroxypropylmethylcellulose (HPMC), Polyethyleneglycol-400 (PEG-400) and methanol were obtained from S.D. Fine

Chem., Chennai. Pre-coated silica gel GF 254 plates were obtained from Merck, USA. Alternative thioglycolate medium (ATGM) and soyabean casein digest medium (SBCD) were obtained from Hi Media, Mumbai. The glass moulds for preparation of ophthalmic inserts were fabricated locally. Timolol Maleate (0.5%) eye drop (Torrent Pharmaceuticals) were purchased from local drug stores. Tonometrics were done by Biro Schiotz Tonometer.

Formulation of ophthalmic inserts

Matrix type ophthalmic inserts of forskolin were prepared by moulding technique. A glass mould of dimensions 10 cm length, 5 cm width and 1.5 cm height with a total surface area of 50 cm² were fabricated for this purpose. Required quantities (125 mg) of the polymers (2.5% sodium alginate and PVA-14000 in the ratio of 1:1) were weighed accurately and to this 10 ml of water were added and heated for sometime till both the polymers become completely soluble. Then 10% w/w of PEG-400 was added followed by addition of 500 mg of powdered extract/ forskolin (the addition of all ingredients, previously sterilised in autoclave at 121°C for 20 minutes at 15 lb, was carried under aseptic conditions to maintain sterility). This mixture was kept for stirring in a magnetic stirrer for 2 hrs. This was allowed to stand for 24 hours and then placed under vacuum to remove the air bubbles. The polymeric drug solution was then poured into pre lubricated glass moulds and kept for drying in a hot air oven at 50°C till complete drying. The films were then cut into 1.0 and 0.5 cm² size pieces. It was finally packed in self sealing poly glassine foils. Thereafter, the inserts were surface sterilised by γ -irradiation at 25 kGy irradiation dose,

for 2-3 minutes and stored in a desiccator for further studies.

Sterility testing

In the present study, two media namely, Alternative Thioglycolate Medium (ATGM) and Soya bean-Casein Digest (SBCD) medium were used to investigate the presence or absence of aerobic, anaerobic bacteria and fungi³², in the formulated sterilized delivery systems.

Preparation of ATGM/ SBCD medium: 7.25 g. of ready made ATGM/ SBCD was dissolved in 250 ml of purified water and the pH was adjusted to 7.1 ± 0.2 with 1M NaOH. The medium was freshly prepared and allowed to cool just prior to use.

Sterilization and testing: Ideal batches of ophthalmic inserts were prepared using moist heat sterilized polymeric solutions under aseptic conditions. After preparation of the inserts, they were surface sterilized by exposing to γ -irradiation for 1, 2, 5 & 10 minutes. All the samples were inoculated separately in to ATGM and SBCD medium and incubated at 37°C and 20-25°C, respectively, for 7 days. Similarly unsterilised ophthalmic inserts samples also inoculated in ATGM and SBCD medium and incubated at 37°C and 20-25°C, respectively for 7 days. A control evaluation was also carried out by the same method. The observations of sterility testing conducted with 6 unsterilised samples and 12 sterilised samples (3 for each time set exposure like 1min, 2min, 5 min and 10 min.) in two different culture media are shown in Table 1 & 2.

***In vivo* Ocular Safety Studies**

A three week (21 days) study was conducted in 30 New Zealand albino rabbits using TED (Timolol Eye Drops), OIE and OIF

ophthalmic inserts were administered and checked for ocular safety evaluation. The ocular safeties of the ophthalmic inserts were done by the Draize test^[38]. The observations based on the scoring approach of ocular tissues like, cornea – opacity (O), area involved (A); Iris – values for congestion and hemorrhage (I) and Conjunctiva – redness (R), chemosis (C) and discharge (D). Those observations were shown in the Table 3.

***In vivo* studies**

The animals were divided into 4 groups. The first was control group, i.e., Ophthalmic Inserts without drug (Control). Three test groups, each comprising of 6 rabbits, one test group TED (Timolol Maleate eye drops) and two groups were administered with Ophthalmic Insert containing Extract of *Coleus forskohlii* (OIE) and Ophthalmic Insert containing Forskolol (OIF). The *in-vivo* studies repeated for three phases by keeping 15 days washout period between two subsequent *in vivo* studies.

Intra Ocular Pressure (IOP) was measured with Schiotz Tonometer, after applying surface anesthesia (Xylocaine 0.5 ml). The tonometrics were usually repeated weekly; too frequent tonometrics were avoided in order to save the integrity of the corneal epithelium. To produce ocular hypertension (steroid inducing glaucoma), the experimental rabbits were treated by subconjunctival injection of 0.2 ml of repository betamethasone³⁹. The subconjunctival injections were repeated weekly for three weeks in different sectors of the eye. Controlled eyes were also injected with the same.

Corneal residence evaluation

In the present study, an effort was therefore made to develop a non-invasive method to

assess the precorneal residence to the formulated drug delivery system based on HPTLC (High Performance Thin Layer Chromatography) technique. A stock solution of forskolin (marker compound) equivalent to 1 mg/ml was prepared and different quantities of this solution namely, 1, 2, 3, 4, 5 μ l were spotted on to precoated silica gel GF plates (10 \times 10 cm size) using automatic sample syringe (Linomat IV Application mode) of the CAMAG-HPTLC equipment in order to develop calibration between 1-4 μ g drug concentrations. The plate was placed in the twin-trough development chamber, which was presaturated with the mobile phase consisting of a mixture of toluene: ethyl acetate (7:3) for OIE & OIF; whereas chloroform: methanol (8:2) for TED, separately. The plate was developed for about 20 minutes time and dried with a current of hot air. The plate was then scanned at 292 nm, (295 nm for TED) in the densitometer and the area under the curve (AUC), of each concentration was determined. Calibration curve was developed by plotting a graph between the concentration and the area under the curve.

Tear sampling and analysis

Tear samples equivalent to 1 μ l were collected from the left eye after application of test delivery system at 0, 0.083, 0.17, 0.25, 0.5, 1, 2, 3, 4, 6, 9, 12 hours post dosing. Glass capillary tubes having 320 μ m internal diameter and 1 μ l premarked were placed near the canthus of the eye without applying pressure. Tear fluid was drained into the tubes due to capillary action and samples equivalent to 1 μ l were mixed with 50 μ l of methanol and spotted onto precoated silica gel GF plates (10 \times 10 cm size). Area under the each

sample curve was determined by developed HPTLC method and the concentrations of forskolin at different time intervals were calculated. The tear fluid concentration-time curves for TED, OIE and OIF were developed and the areas under their curves were calculated adopting trapezoidal rule. Results are shown in Fig. 1.

Pharmacodynamic studies

The potential of formulated drug delivery systems of forskolin in controlling the IOP was evaluated by adopting tonometric technique³⁹. IOP measurements were done at 0, 0.5, 1, 2, 3, 4, 6, 8, 9, 12, 16 & 24 hours post administration. Schiottz tonometer was cleaned with spirit and placed in a convex test block to assure zero position of the pointer. The weight marked 5.5 g. was always on the plunger. For measuring the IOP, rabbits were placed in restraining boxes and eyelids were retracted gently with one hand, without exerting pressure on the eye ball and the tonometer was placed in the horizontal position on the centre of the cornea. The handle was in the midway between the top and foot plate of the cylinder, thereby the instrument might cut the independently with its own weight. The position of the pointer was noted and the tension in mm Hg was determined from the calibration scale. The observations of pattern of IOP and the changes in IOP were calculated. Results are shown in Fig. 2 and 3.

Statistical analysis

Data are presented as mean \pm S.E.M. Statistical significance of the data for control and treated groups was assessed by One-Way Analysis of Variance (ANOVA GraphPad InStat Software). Statistical significance was accepted when $p < 0.001$ (extremely

significant by Bonferroni Multiple Comparisons Test).

RESULTS

Microbial growth was observed in all the unsterilised samples in both ATGM and SBCD. However, no growth was observed in ophthalmic inserts prepared by using moist heat sterilized polymeric solutions, and subsequently followed by ophthalmic inserts surface sterilization by γ -irradiation for more than 2 minutes. Based on these observations, moist heat sterilization of polymeric solutions and surface sterilization by γ -irradiation at 25 kGy, of inserts for 2 minutes after preparation of inserts, were ideal for achieving sterility.

The ocular safety observations for the formulated ophthalmic inserts in the rabbit eyes adapting Draize's scoring approach. The ocular safety score for the OIE and OIF Inserts was found to be 0.33. These safety scores are very insignificant when compared to the maximum score of 110 (by evaluation chart). There was no adverse reaction, redness, on cornea, iris and conjunctiva, after application of the formulated ophthalmic inserts. Thus, the formulated ophthalmic insert is non-irritating to eye. It can be concluded that the ophthalmic inserts were safe for ocular administration in rabbit's eye.

The precorneal residence of forskolin after application of equivalent doses containing ophthalmic inserts in rabbit eyes. There was a significant improvement in precorneal residence of forskolin after application of the formulated drug delivery systems. Measurable tear fluid levels were noted from 0.5 hour onwards. In case of the ophthalmic inserts prepared, the levels were maintained for 12 hours for OIF; but pharmacologic effect for 24 hours of decreased IOP and 12

hours for OIE. The increase in corneal residence may be attributed to the mucoadhesive nature of the ophthalmic inserts and the formation of a gel like consistency after certain period of time.

The areas under tear fluid concentration–time curve for the formulated ophthalmic inserts were studied. Based on these areas, an interpretation can be made to determine the effectiveness of the ophthalmic inserts for reducing the intraocular pressure. The AUC for the, OIF was found to be higher than that of OIE. Hence, it can be stated that OIF inserts have better efficacy than OIE inserts.

Pharmacodynamic evaluation was performed to interpret the efficacy of the developed formulation and the drug. After the application of ophthalmic inserts, there was a reduction in IOP. The OIF, showed a reduction of 15.6 mm Hg in IOP in 9 hours time period and the OIE, showed a reduction of 13.1 mm Hg IOP in 4 hours. The activity was noticed over a period of 24 hour post administration for OIF and 12 hour post administration for OIE. This control in the IOP for prolonged periods may be attributed to the increased corneal residence and sustained drug release potential of the formulated ophthalmic inserts.

Table 1 : Sterility test observations in ATGM medium of OIE & OIF after γ -irradiation.

Samples	Days							
	1	2	3	4	5	6	7	30
Control	–	–	–	–	–	–	–	–
Unsterilized (OIE & OIF)	–	√	√	√	√	√	√	√
Surface sterilized (OIE & OIF)								
1 min	–	–	√	√	√	√	√	√
2 min	–	–	–	–	–	–	–	–
5 min	–	–	–	–	–	–	–	–
10 min	–	–	–	–	–	–	–	–

(–) Absence of microbial growth; (√) Presence of microbial growth.

Table 2 : Sterility test observations in SBCD medium of OIE & OIF after γ -irradiation.

Samples	Days							
	1	2	3	4	5	6	7	30
Control	–	–	–	–	–	–	–	–
Unsterilized (OIE & OIF)	–	√	√	√	√	√	√	√
Surface sterilized (OIE & OIF)								
1 min	–	–	√	√	√	√	√	√
2 min	–	–	–	–	–	–	–	–
5 min	–	–	–	–	–	–	–	–
10 min	–	–	–	–	–	–	–	–

(–) Absence of microbial growth; (√) Presence of microbial growth.

Table 3 : Ocular safety scores of OIE and OIF.

Group	Day	Mean recorded score						Safety score						Total score	Safety rating
		Cornea		Iris		Conjunctiva		Cornea		Iris		Conjunctiva			
		O	A	I	R	C	D	O	A	I	R	C	D		
OIE	1	-	-	-	-	-	-	-	-	-	-	-	-	0.33	Non-irritating
	3	-	-	-	-	-	0.15	-	-	-	-	-	0.33		
	6	-	-	-	-	-	-	-	-	-	-	-	-		
OIF	1	-	-	-	-	-	-	-	-	-	-	-	-	0.33	Non-irritating
	3	-	-	-	-	-	0.15	-	-	-	-	-	0.33		
	6	-	-	-	-	-	-	-	-	-	-	-	-		

Fig. 1 : Tear fluid concentration-Time curve for TED, OIE & OIF.

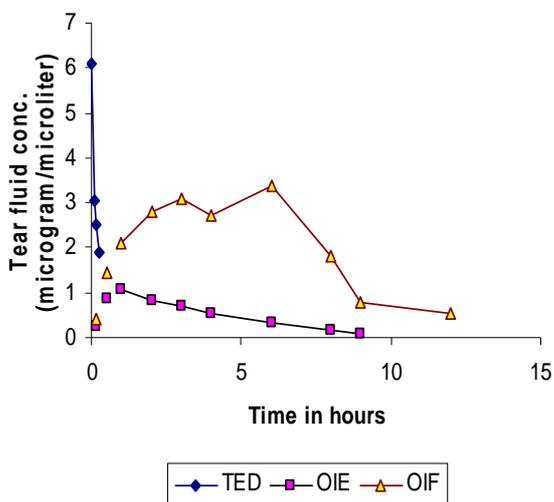


Fig. 3 : Change in IOP after application of TED, OIE & OIF.

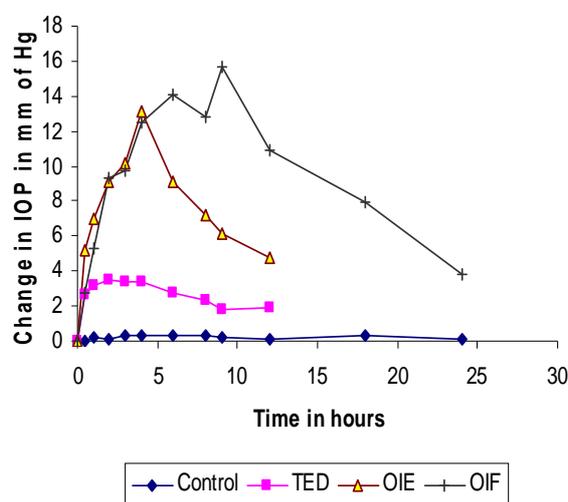
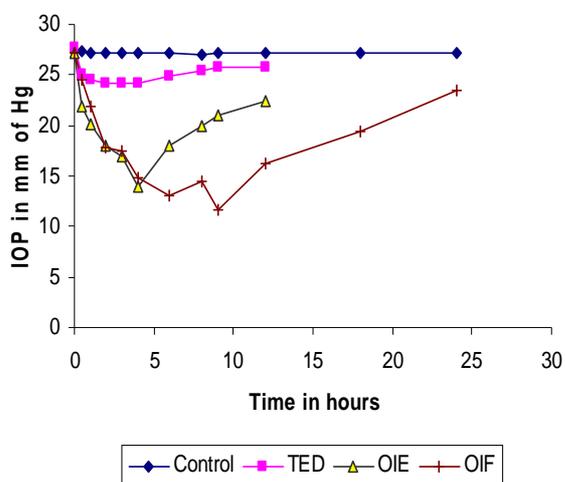


Fig. 2 : Pattern of IOP after application of TED, OIE & OIF.



DISCUSSION

The review of literature has shown that the forskolin has an intra oculohypotensive effect and hence useful in the treatment of the glaucoma. The ideal Class-I, antiglaucoma agent reducing IOP, by 25-30 %. The forskolin is found to reduce the IOP by 23-28% (instilled as 1% solution). So definitely it is a suitable antiglaucoma agent. The reported eye drop formulation was done by Hoechst research. But, the effort to forskolin ophthalmic inserts was remaining. The same hypothesis we tried to work out here.

The studies embodies ocular safety studies, pharmacodynamic efficacy and precorneal residence time, were evaluated in New Zealand albino rabbit eyes to investigate their acceptance and suitability for ocular administration. Our efforts to formulate and develop forskolin ophthalmic inserts intended at improving the corneal bioavailability of forskolin brought out various observations which lead to the following conclusions.

Moist heat sterilization of the polymeric solution, followed by surface γ -irradiation sterilization at 25 kGy, for more than 2 minutes is suitable to achieve sterility of the ophthalmic inserts. The developed ophthalmic inserts were found to be safe for ocular administration and capable of residing in the precorneal area of the eye for extended periods of time. Moreover, these were very effective in controlling the IOP for 24 hours as deduced from the *in vivo* animal studies conducted.

Forskolin has a different molecular mechanism from any previously used antiglaucoma drug. Its effect on IOP should be additive with other drugs because it has unique mode of action, and tachyphylaxis might not occur because forskolin's action is not believed to involve the cell surface receptors. Present results suggest the significant reduction in IOP by Ophthalmic Inserts Drug Delivery System (OIDDS), of forskolin. Therefore, it may be useful in the treatment of a glaucomatous eye of glaucoma suffering patients.

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