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RESEARCH ARTICLE

In Vivo studies of Ophthalmic Ocular Insert Containing Aciclovir

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ABSTRACT:
Aciclovir is an antiviral drug used to treat viral infections like herpes simplex virus. The aim of the present research work was to develop ocular inserts of aciclovir and evaluate their drug release for both *in vitro* and *in vivo*. The draw backs of other ophthalmic formulations (solutions, suspensions, ointment) like poor bioavailability, patient non compliance, quick drug elimination was overcome by formulating the drug in ocusert formulation.[1,2] The ocuserts was prepared by solvent casting technique using hydrophilic and hydrophobic polymers. Eight formulations was prepared with formulation code of F1, F2, F3, F4, F5, F6, F7, F8.The satisfied drug release was achieved in F2 formulation. The selected formulation F2 was subjected to sterilization before performing *in vivo* studies.[3,4]6 healthy rabbits are used for in vivo study. Draize irritation studies are carried out to check the compatibility of ocusert in the eyes. After 21 days of irritation study the eyes are examined. Drug content of the ocuserts are calculated at predetermined time intervals and subtracted from the initial value.[5]Theinvivo drug release results are correlating with *invitro* drug release, which shows better therapeutic efficacy.

KEYWORDS: Aciclovir, *in vivo*, Draize irritation, Rabbits, drug content.

INTRODUCTION:
Eye is an important organ in the human body. Aciclovir drug is having half life of 2-4 hrs which is necessary for controlled release formulation.[1]The factors to be considered while formulating controlled drug release was shown in table no 1

Table No 1 Properties of drug for controlled release formulation

S.No	Properties of drug	Limits allowed
1	Molecular weight	Less than 600 Daltons
2	Partition coefficient	1-2
3	Elimination half life	2-6 hrs

Mechanism of action aciclovir is shown in the Fig 1

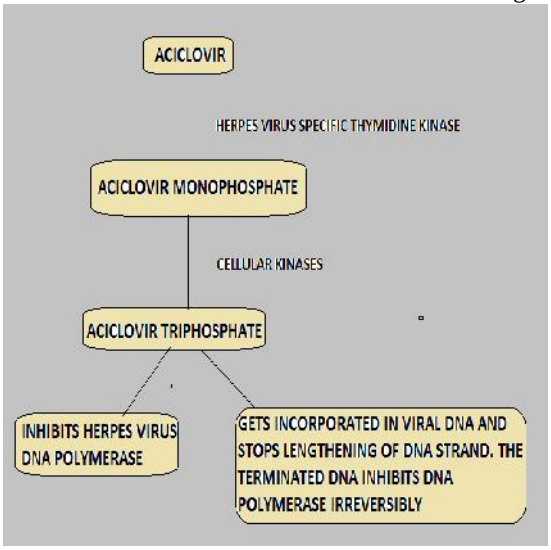

Fig No 1 Mechanism of action of aciclovir

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Criteria for controlled release ocular insert are comfort, convenient to use, sterility, stability, easy to formulate, excipients used non irritant to the eyes

MATERIALS AND METHODS:

Aciclovir was received as a gift sample from Kaushik Therapeutics Pvt. Ltd. Chennai. The polymers hydroxy propyl cellulose, Polyethylene glycol 400, Eudrajit RS 100, Ethyl cellulose was purchased from Nice Pharmaceuticals, Kerala. All other ingredients were of analytical grade.

Preparation of ocular inserts:

Preparation of drug reservoir[7,8]:

Polymeric solutions were prepared by dissolving hydrophilic polymer HPC/PVA along with aciclovir and polyethylene glycol 400 in doubly distilled water. The solutions were poured into a glass ring of 8.9 cm diameter placed in a Teflon coated petridish. The solvent was allowed to evaporate by placing it inside an oven maintained at 35 $^{\circ}$C for 24 hrs. The formulation of aciclovir ocular insert was shown in table no 2

Preparation of rate controlling films:

To prepare the rate controlling films hydrophobic polymer (ethylcellulose, eduragit RL 100) along with plasticizer Dibutyl phthalate was dissolved in ethanol/acetone (80:20). The solutions were poured into a glass ring 8.9 cm diameter petriplate. The solvent was allowed to evaporate at 25°C for 24 hrs. Placing rate controlling film around, the drug reservoir and sealing them to obtain ocular inserts. The two rate controlling membranes containing the reservoir film between them were placed over a beaker saturated with ethanol/acetone vapors 60:40 for two minutes. The ophthalmic ocusert formulation is shown in Fig no 2

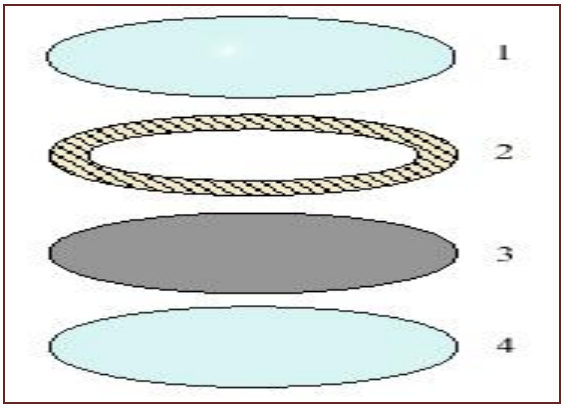

Fig No 2 Different layers of ocuserts

1. Transparent polymer membrane 2.Polymeric membrane ring 3. Drug reservoir 4.Transparent polymer membrane.

Table No:2 Formulation of aciclovir ocular inserts

In gredients	F1	F2	F3	F4	F5	F6	F7	F8
PREPARATION OF DRUG RESERVOIR								
Aciclovir*	10	10	10	10	10	10	10	10
HPC*	2		-	-	2	4	-	-
PVA**	-	-	2	4	-	-	2	4
PEG 400**	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Distilled water	10	10	10	10	10	10	10	10
Preparation Of Rate Controlling Membrane								
Ethyl cellulose*	4	2	4	2	-	-	-	-
Eudragit RL 100*	-	-	-	-	4	2	4	2
PEG400**	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Acetone	10	10	10	10	10	10	10	10

*Quantity in %**quantity in ml

***In-vitro* diffusion studies:**

Studied using standard open ended cylindrical glass tube. The pre hydrated cellophane was tied to one end of cylindrical tube which act as a donor compartment. An ophthalmic insert was placed inside the donor compartment. The receptor compartment containing 25 ml of simulated tear fluid in 100 ml beaker. The content of the receptor compartment was stirred using magnetic stirrer at 37^0C.±0.5 ^{0}C .At specific time interval 1ml of simulated fluid from receptor compartment was withdrawn and replaced with fresh simulated tear fluid. The withdrawn samples are diluted and analyzed using UV at 254 nm.

Drug content uniformity:

Ocular inserts were dissolved individually in methanol/ethanol in a 100 ml volumetric flask. Then required volume of solution was taken out and further dilutions were made with STF pH 7.4. Similarly, a blank was carried out using a drug free insert. Then absorbance was taken at 254.0 nm by UV spectroscopy.

***In vivo* drug release studies:**

Eye irritancy Test (Draize eye irritancy test) [8]:

The selected formulations F2 were sterilized by UV radiation before performing animal studies. The institutional animal ethical committee (IAEC) approval was obtained before performing *invivo* drug release with the reference No: XIX/VELS/P.COL/12/2000/ CPCSEA/IAEC/O3.10.2016 .Eye irritation test was done to detect the damage of cornea, iris and conjuctivia caused by the formulation in the eye.

Animal: Rabbits
Species: Albino
Gender: Male
Number of animals: Six

Housing:
The rabbits were housed in standard cages, in a light controlled room at 28 ± 2°C and 60 ± 15 % relative humidity, with no restriction of food or water.

During the experiments, the rabbits were placed in restraining boxes, where they could move their eyes and head freely. Selected sterilized ocusert was instilled in cul-de-sac of right eye and left eye kept as control. Both eyes were periodically observed by naked eye or by means of a pen torch for Redness, Swelling andwatering of the eye. Test eye (right eye) will be compared with control eye (left eye).

Table no: 3 Ocular irritation scoring system

S. No	Eye part	Ocular reaction	Score
1	Cornea	No ulceration	0
2		Scattered or diffuse area	1
3		Easily discernible translucent area	2
4	Iris	Normal	0
5		Swelling	1
6		Hemorrhage	2
7	Conjuctiv	Blood vessels are normal	0
8	ia	Blood vessels are hyperemic	1
9		Diffuse crimson color	2

This test was repeated for 21 days and finally reported as ocular safety of the selected formulation. At the time of examination period each rabbit was scored for ocular reaction given in Table 3. The test may be considered positive if three or more animal exhibit positive reactions at any observation period.

***In vivo* drug release studies [9 10]:**
Six healthy rabbits were used to study the *in vivo* drug release studies. The rabbits used for the *in vivo* study was kept in good condition in order to exclude any diseases. The selected ocuserts are placed in cul-de-sac of each rabbit and another eye served as a control. At predetermined time intervals (2, 4, 6, 12 and 24 hrs) the inserts were taken out carefully from the eyes of each rabbit and analyzed for the remaining drug content. The drug content remaining was subtracted from the initial drug content of the insert, which gives the amount of drug released at the specific time in the rabbit eye.

RESULTS AND DISCUSSION:

***In vitro* diffusion studies[7,2,3]**
The F2 formulation containing Hydroxypropylcellulose (4mg) in the drug reservoir and Ethylcellulose (2mg) was showing satisfied controlled release when compared to remaining formulations. After 24 hrs the percentage drug release from F2 formulation was found to be 97.16%.The percentage drug release is shown in Table 4.

Table No 4 *In vitro* drug release studies of formulated ocular inserts

S. No	Time in hrs	F1	F2	F3	F4	F5	F6	F7	F8
1	0	0	0	0	0	0	0	0	0
2	2	18.1±0.9	10.0±0.9	8.3±0.1	22.8±0.4	20.0±0.8	33.3±0.9	33.3±0.8	37.5±0.7
3	4	30.6±0.2	20.8±0.8	30.6±0.2	33.3±0.2	39.4±0.8	66.7±0.5	55.6±0.8	56.7±0.3
4	6	50.0±03	43.3±1.2	51.7±0.5	45.6±0.2	58.3±0.3	79.2±0.3	77.8±0.5	77.2±0.8
5	8	55.0±0.1	61.1±0.6	54.4±0.4	56.1±0.1	77.8±0.2	82.7±0.7	82.0±0.4	90.5±0.4
6	12	69.4±02	77.8±0.3	72.2±0.3	71.4±0.2	80.5±0.4	83.8±0.4	96.1±0.2	93.4±0.2
7	24	80.6±0.5	97.16±0.1	82.8±0.2	83.9±0.1	82.0±0.2	93.3±0.6	96.13±0.2	94.7±0.2

All the values are expressed as mean ±SD, n=3

***In vivo* Study:**
Eye Irritation Test[5]:
The selected formulation F2 which shows promising *in vitro* drug release was subjected to eye irritation test after sterilized by UV radiation. The results shows that there was no irritation, redness of eye, and swelling of eyes .so it was concluded that the prepared ocular inserts were compatible to the eyes. The ocular inserts was not expelled from the eye during eye irritation study. The ocusert insertion in to rabbit eye are shown in Figures 3,4,5,and 6.As the prepared inserts does not show any inflammation and abnormal discharge the excipients used in the formulation also found to be compatible with the eye. The eye irritation test results are shown in table no 5

Table No 5 Results of eye irritation study

S. No	PART OF EYE	SCORING VALUE
1	Cornea	0
2	Iris	0
3	Conjuctivia	0

Fig No 3 Normal rabbit eye

Fig No 4 Inserting ocusert in to rabbit eye

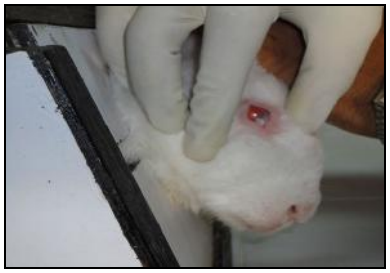

Fig No 5 Ocusert inserted in rabbit eye

Fig No 6 Rabbit eye after irritation study

In vivo drug release study:

After obtaining approval from institutional animal ethical committee (IAEC) under Ref No. XIX/VELS/P.COL/12/2000/CPCSEA/IAEC/O3.10.2016 the animal studies were carried out. The *in vivo* studies were performed using healthy rabbits. The drug content of the inserts was calculated at specific time intervals. The drug release was found to be as shown in table no 4.The *in vitro* drug release was correlating with *in vivo* drug results. The *in vivo* drug release results are shown in table no.6. Graphical representation of *in vivo* drug release was shown in Fig No 7

Table No 6 *In vivo* drug release for F2 formulation

S. No	Time intervals in hrs	In vivo % Drug release
1	0	0
2	2	9.6±0.9
3	4	19.5±0.7
4	6	42.5±0.9
5	8	60.5±0.5
6	12	76.2±0.6
7	24	98.5±0.2

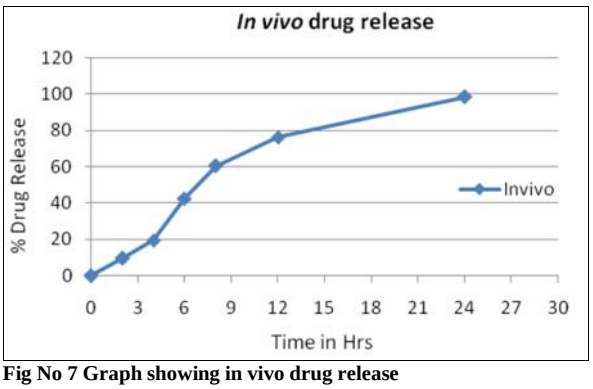

Fig No 7 Graph showing in vivo drug release

CONCLUSION:

The ocular inserts were prepared by solvent casting technique by enclosing the drug reservoir membrane in between two rate controlling membranes. The drug reservoir was made up of hydrophilic polymer and the rate controlling membrane was made up of hydrophobic polymer. Eight formulations containing different ratios of polymers were prepared. The *in vitro* drug release results shows that F2 was the best formulation containing Hydroxypropylcellulose 4mg and Ethylcellulose 2mg, The F2 formulation meets all the controlled release formulation criteria's .The selected formulation F2 was subjected to eye irritation study and *in vivo* study. The formulation passes Draize eye irritation test as there was no swelling, redness of the eye. The *in vivo* results are correlating with *in vitro* results, so the prepared formulation F2 shows better therapeutic efficacy.

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REFERENCES:

1. Bagool M.A. Topical ocular drug delivery a review. *Indian Drugs*, 1993, 31(10), 451-456.
2. Dandagi P.M. Development and evaluation of ocular films of Cromolyn sodium. *Indian J. Pharm. Sci.*, 2004, 66(3), 309-312.
3. Dandagi P.M., Manvi F.V., Gadad A.P. and Wagh B.P. Development and evaluation of Ketorolac tromethamine ocular films. *Indian Drugs*, 2003, 40, 369-371.
4. Dhanaraju M.D., Sivakumar V.R. and Bhaskar K. Bioadhesive ocuserts matrix for ophthalmic administration of Ciprofloxacin hydrochloride. *Indian Drugs*, 2002, 39, 222-224.
5. Draize J.H., Woodward G. and Calvery H.O. Methods for the study of irritation and toxicity of substance applied topically to the skin and mucous membrane. *J. Pharmacol. Exp Ther.*, 1944, 82, 377-390.
6. Gandra R., Khatry S., Shastri N. and Sadanadam M. Formulation and evaluation of ocular inserts of Moxifloxacin hydrochloride. *Indian Drugs*, 2009, 46(3), 214-220.
7. Gupta A., Sharma S.K. and Ahuja M. *In-vitro* and *in-vivo* evaluation of Gellan based ocular inserts of Phenylephrine. *Acta Pharmaceutica Sciencia*, 2007, 49, 55-63.
8. Jain N.K. Controlled and novel drug delivery, 2008, CBS publishers, New Delhi, 82-99.
9. Jain N.K., SulekhaBhadra and Dipankar Bhadra. Pharmaceutical product development, 2008, CBS publishers, 182-210.
10. Jayaprakash S., James C.C., Gerald Rajan, Saisivam S. and Nagarajan M. Design and evaluation of Ketorolac tromethamine ocuserts. *Indian J. Pharm. Sci.*, 2000, 62(5), 334-338.