AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Transcorneal Permeation of Ketrolac Tromethamine by Amino Acid Transporters

Ugandar R E[1]*, Dinesh Kumar Sharma[2], Darsiga Selvarajah[3], Kiran C Nilugal[3]

1. Pacific Academy of Higher Education and Research, Pacific University, Udaipur, Rajasthan India.

2. Devasthali College of Pharmacy, Uttar Khand, India.

3. *Faculty of Pharmacy, Asia Metropolitan University, Batu 9, Cheras, 43200 Selangor, Malaysia.*

ABSTRACT

The research involve formulation and evaluation of three different ophthalmic solutions of Ketorolac tromethamine with three different amino acid transporters. The prepared formulations were subjected to ex-vivo transcorneal permeation and cornea hydration studies through excised goat cornea and the results were compared with those of a prepared standard Ketorolac tromethamine ophthalmic solution. The trans corneal permeation studies were carried out by using an all glass Franz diffusion cell. The non-steroidal anti-inflammatory drug Ketorolac tromethamine ophthalmic solution is indicated for the temporary relief of ocular itching due to seasonal allergic conjunctivitis and also for the treatment of inflammation following cataract surgery. In the study, the three amino acid transporters used were Lysine, Phenylalanine and Valine which are responsible for protein synthesis and play a significant role in the process of maintaining structural and functional integrity of conjunctiva and retina. The results obtained from the permeation studies of conjugated physical mixtures of Ketorolac tromethamine with different amino acid transporters across the excised goat cornea were observed to exhibit enhanced permeation when compared with the permeation of Ketorolac tromethamine from the standard formulation.

Keywords: *Ex-vivo*, corneal permeation, conjugation, transporters and Ketorolac Tromethamine.

*Corresponding Author Email: reugandar@gmail.com

Received 28 July 2015, Accepted 03 August 2015

Please cite this article as: Ugandar RE *et al*., Transcorneal Permeation of Ketrolac Tromethamine by Amino Acid Transporters. American Journal of PharmTech Research 2015.

INTRODUCTION

One of the most complex organs in the human body is the eye. The eye can be illustrated as being comprised of three different regions: the outer cornea and sclera; the middle layer, which consists of the iris, ciliary body, and the choroid; and the inner region or retina[1]. The cornea is a transparent tissue that is dependable for the refraction of the incoming light and is multi layered tissue made up of three major cell layers which are the epithelium, the stroma and the endothelium. Due to the dual nature of the cornea, with a lipophilic epithelium and a hydrophilic stroma, the epithelium show to be rate limiting to the movement of hydrophilic compounds while the stroma is the rate limitating for lipophilic compound[2]. Furthermore, most of the drug is washed out from the ocular surface by numerous mechanisms such as lacrimation, tear dilution, and tear turnover before they are able to penetrate into the desire tissue[3].Delivery of medication to the human eye is an integral part of medical treatment. Ocular drug delivery is one of the most fascinating and challenging endeavours of the pharmaceutical scientists. The eye is highly impervious to foreign substance due to anatomy, physiology and biochemistry of this organ. To get out of protective barrier of the eye without producing permanent tissue damage is the major challenge to the formulator. Ocular delivery system with high therapeutic efficiency is continuously being achieved by development of newer, more sensitive diagnostic techniques and novel therapeutic agents. Recently, the most appealing approach to improve transcorneal permeability of hydrophilic moieties appears to be targeted drug delivery by means of amino acid transporters. Drug- amino acids were conjugated and these were used to enhance transcorneal permeability of drug. The most effective method for drug targeting is believed to be the amino acids and peptide transporter as these transporter have enormous range of substrates and direction of transport from epithelium to endothelium providing a possible task in the permeation of substrate molecule[4].The presences of various amino acid transporters such as LATI, ATB^{0+} and ASCTI in cornea have been proved. These transporter are involved in the transport of several amino acids such as L-lycine, L-phenylalanine and L-valine across the cornea[5]. Ketorolac Tromethamine is a member of the pyrrolo-pyrrole group of Nonsteroidal Anti -Inflammatory drugs. Ketorolac, 5-benzoyl-2, 3-dihydro-1H-pyrrolizine-1-carboxylic acid, is a racemic mixture. The anti-inflammatory activity of the levorotatory (*l*) isomer of the drug is twice that of dextrorotatory isomer. It is commercially available as the tromethamine salt which has higher aqueous solubility compared to ketorolac. Ketorolac tromethamine ophthalmic solution is indicated for the temporary relief of itching due to seasonal allergic

conjunctivitis and also treatment of postoperative inflammation in patients who were undergone cataract extraction[6].

MATERIALS AND METHODS

Chemicals:

Ketorolac tromethamine was obtained as gift sample from Y.S.P. Industries and the other chemicals used were Amino acids (L-Lysine, L-Phenylalanine, L-Valine), Benzalkonium Chloride, Edetate Disodium (EDTA), Sodium Chloride, Hydrochloric Acid, Sodium Hydroxide, Distilled water, Ethanol 50% , Silica gel, Normal saline (0.9% Sodium chloride), Butan-1-ol- analytical method, Acetic acid –analytical method, Sodium bicarbonate, Calcium chloride and Potassium chloride.

Isolated animal organ:

Fresh Whole Goat's Eye Ball.

Equipment's:

The equipments used include Weighing balance, Dissection kit, Hot plate, Desiccator, pH meter, Franz diffusion cell, Rotary Evaporator, Thermometer, Thin layer chromatography plate, Hot air oven, Ultraviolet-visible spectrophotometer, Aluminium foil, Teflon coated magnetic stirrer,

Formulation Methods

Synthesis of Ketorolac Tromethamine and Various amino acid conjugates

Formulations of three different ophthalmic solutions of Ketorolac tromethamine were prepared by dissolving the conjugated mixture of Ketorolac and an amino acid in the vehicle. The conjugates were prepared by Solvent Evaporation technique where the Ketorolac tromethamine and amino acid conjugates prepared by dissolving equimolar amounts of the drug and the corresponding amino acid in ethanol 50% (v/v) under short time heating not exceeding 50^{o}C. The solvent was evaporated under vacuum conditions by using rotary evaporator. The white to off-white crystalline substances formed were dried in a dessicator over silica gel for one week[5].

Characterization of the synthesized compound

Visual observation of conjugates was done with respect to colour, odour and nature of the conjugates. The conjugates were subjected to thin layer chromatography in order to check their purity. The prepared plates of silica gel G adsorbents were dried and activated. The solvent system butanol, acetic acid and water in the ratio 8:2:2 by volume was used for amino acids. Ninhydrin reagent was used as detecting agent[7].

Preparation of standard solution

Ketorolac tromethamine ophthalmic solutions (0.5% w/v)

Ketorolac tromethamine (500mg) was dissolved in sufficient distilled water; sodium chloride was added to make the final solution isotonic; pH was adjusted to 7 using 0.1N HCL and 0.1N NaOH respectively and final volume was made up to 100ml with distilled water.

Preparation of test solutions

Ketorolac tromethamine and Valine conjugates ophthalmic solution (0.5% w/v)

Conjugated Ketorolac tromethamine with Valine (656mg) was dissolved in sufficient distilled water, sodium chloride was added to make final solution isotonic. pH of the solution was adjusted to 7.0 using 0.1N HCL and 0.1N NaOH and final volume was made up to 100ml with distilled water to have solution of (0.5%w/v) drug amino acids conjugate of Ph 7.0.

Ketorolac tromethamine and Phenylalanine conjugate ophthalmic solution (0.5% w/v)

Conjugated Ketorolac tromethamine with Phenylalanine (720mg) was dissolved in sufficient distilled water, sodium chloride was added to make final solution isotonic pH of the solution was adjusted to 7.0 using 0.1N HCL and 0.1N NaOH and final volume was made up to 100ml with distilled water to have solution of (0.5%w/v) drug amino acids conjugate of Ph 7.0.

Ketorolac tromethamine and Lysine conjugate ophthalmic solution (0.5% w/v)

Conjugated Ketorolac tromethamine with Lysine (694mg) was dissolved in sufficient distilled water, sodium chloride was added to make final solution isotonic. pH of the solution was adjusted to 7.0 using 0.1N HCL and 0.1N NaOH and final volume was made up to 100ml with distilled water to have solution of (0.5%w/v) drug amino acids conjugate of Ph 7.0.

Preparation of eye drop of test solutions

Eye drops were prepared by dissolving the required quantity of excipients in distilled water. The ingredients are EDTA disodium, sodium chloride and benzalkonium chloride. All the formulations were prepared and pH was adjusted by using 0.1N HCL/0.1N NaOH. Then, permeation studies of eye drops were carried out. The table below shows the amount of drug and excipients in each formulation.

Table 1: Ingredients and quantities of Standard Ketorolac Tromethamine formulation

Product Code	Ingredients	Quantity(mg)	Purpose
KT (Standard formulation of Ketorolac Tromethamine)	KT	500	Active ingredient
	NaCl	790	For isotonicity
	Benzalkonium Chloride	10	Preservative
	EDTA	100	Chelating agent
	HCl (0.1N)	q.s.	pH adjuster
	NaOH (0.1N)	q.s.	pH adjuster
	Distilled Water	q.s.to100ml	Solvent

Table 2: Ingredients and quantities of Ketorolac Tromethamine and amino acid conjugate test formulations

Product Code	Ingredients	Quantity (mg)	Purpose
KTL (Formulation of Ketorolac Tromethamine and Lysine conjugates)	KTL	694.19	Active ingredient
	NaCl	790	For isotonicity
	Benzalkonium Cl^-	10	Preservative
	Disodium EDTA	100	Chelating Agent
	HCl (0.1N)	q.s.	pH adjuster
	NaOH (0.1N)	q.s.	pH adjuster
	Distilled Water	q.s.to 100ml	Vehicle
KTP (Formulation of Ketorolac Tromethamine and Phenylalanine conjugates)	KTP	719.5	Active ingredient
	NaCl	790	For isotonicity
	Benzalkonium Cl^-	10	Preservative
	Disodium EDTA	100	Chelating Agent
	HCl (0.1N)	q.s.	pH adjuster
	NaOH (0.1N)	q.s.	pH adjuster
	Distilled Water	q.s.to 100ml	Vehicle
KTV (Formulation of Ketorolac Tromethamine and Valine conjugates)	KTV	655.5	Active ingredient
	NaCl	790	For isotonicity
	Benzalkonium Cl^-	10	Preservative
	EDTA	100	Chelating Agent
	HCl (0.1N)	q.s.	pH adjuster
	NaOH (0.1N)	q.s.	pH adjuster
	Distilled Water	q.s.to100ml	Vehicle

Preparation of stock solution of Ketorolac Tromethamine

50 mg of standard Ketorolac Tromethamine was weighed and transferred to 100 ml volumetric flask. Ketorolac Tromethamine was dissolved in 35 ml methanol by gentle shaking and volume was made up to the mark with same solvent to obtain final concentration of 500 µg/ml and labeled as 'Dilution-1'. From the 'Dilution-1' solution 2 ml of aliquot was pipetted out in to a 25 ml volumetric flask and the volume was made up to the mark with methanol to obtain final concentration of 40 µg/ml and labeled as 'Dilution-2'[9].

Preparation for Calibration Curve of Ketorolac Tromethamine

For the quantitative measurements of Ketorolac Tromethamine, from the 'Dilution-2' (40 µg/ml) solution 1.0, 1.5, 2.0, 2.5 and 3 of aliquots were pipetted out in to series of 10 ml volumetric flasks and the volumes were made up to the mark with methanol to obtain the concentration of 4, 6, 8, 10 and 12µg/ml. Absorbance of the above solutions (3-13 µg/ml) were measured at 322 nm and a calibration curve was constructed by plotting absorbance *vs* concentration graph[10].

EVALUATION METHODS

pH of the eye drops

Eye drop was evaluated for its pH, and visual appearance. The pH was determined by using pH meter

***Ex- vivo* transcorneal permeation studies**

The whole eye ball was transported from a local butcher shop to the laboratory in cold (0.4^{o}C) normal saline (0.9% NaCl) within 1 hour slaughtering the animal. The cornea was carefully excised along with 2-4mm of the surrounding sclera tissue portion remaining adhered to the cornea for ease of mounting and the cornea was washed with cold normal saline till the washing as free from proteins. The cornea was mounted by sandwiching the surrounding sclera tissue between clamped donor and receptor compartments of an all glass modified Franz Diffusion cell in such a way that its epithelial surface faced the donor compartment. The area of diffusion cell was 0.85cm^2. The receptor compartment was filled with 10ml of freshly prepared bicarbonate ringer solution and ensured that no air bubble was present in the compartment. A 1 ml aliquot (5000 µg/ml) of test formulation was placed on the cornea and opening of the donor compartment was sealed with an aluminium foil to prevent evaporation. The receptor fluid was maintained at 37^{o}C with constant stirring using a Teflon coated magnetic stir bead. Permeation study was continues for 120 minutes and at various time intervals 3 ml samples were withdrawn from the receptor compartment and immediately replaced with equal volume of bicarbonate ringer solution.

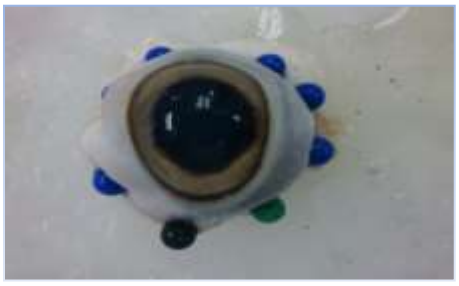

Figure 1: Goat's fresh eye ball pinned on dissection board

Figure 2: Excised cornea along with 2-4mm of sclera tissue

Figure 3: Fixing of excised cornea to the Franz diffusion cell

Figure 4: The receptor compartment was filled with bicarbonate ringer solution

Figure 5: Final setup of the Franz diffusion cell with a teflon coated magnetic bead in receptor compartment and the cell was maintained at 37^0C with a beaker containing distilled water on a hot plate.

Analytical method

Each experiment was continued for about 2.0 hours and from the three ml of withdrawn samples, one ml was analyzed in UV spectrophotometer for Ketorolac Tromethamine content by measuring absorbance at 322nm. The results were expressed as amount permeated and % Permeability. The % permeability was calculated as follows:

$$\textbf{Permeability}\ (\%) = \textbf{100} \times \frac{\textbf{Amount permeated in receptor}}{\textbf{Initial amount of drug in donor}}$$

At the end of experiment, each cornea (freed from adhering sclera) was weighed, soaked in 1ml methanol, dried overnight at 90°C and reweighed. From the difference in weight, corneal hydration was calculated. Hydration was calculated by the following formula:

$$\textbf{Hydration}\ (\%) = \textbf{100} \times \frac{\textbf{Wd}}{\textbf{Ww}}$$

Where W_d =Weight of dried cornea and

W_W = Weight of wet cornea.

RESULTS AND DISCUSSION

Table 3: Quantities of Ketorolac Tromethamine and amino acids used for conjugation

Product code	Quantities of Ketorolac Tromethamine and various amino acids in equimolar amounts				Ethanol (50%) (ml)
	Ketorolac Tromethamine (gm)	Lysine (gm)	Phenylalanine (gm)	Valine (gm)	
KTLC	5.0	1.942	-	-	q.s.
KTPC		-	2.194	-	
KTVC		-	-	1.556	

Table 4: Observations of physical characterization of Ketorolac Tromethamine and amino acid conjugates

Characterization	Ketorolac Tromethamine with various amino acids		
	Lysine	Phenylalanine	Valine
Colour	White	White	White
State	Crystalline	Crystalline	Crystalline
Odor	Odorless	Odorless	Odorless

Confirmation of Conjugation using TLC[10,11]

Figure 6: TLC of Ketorolac Tromethamine and Valine Conjugates

K: Ketorolac Tromethamine V : Valine C: Conjugate of Drug and Valine

Preparation of Calibration curve

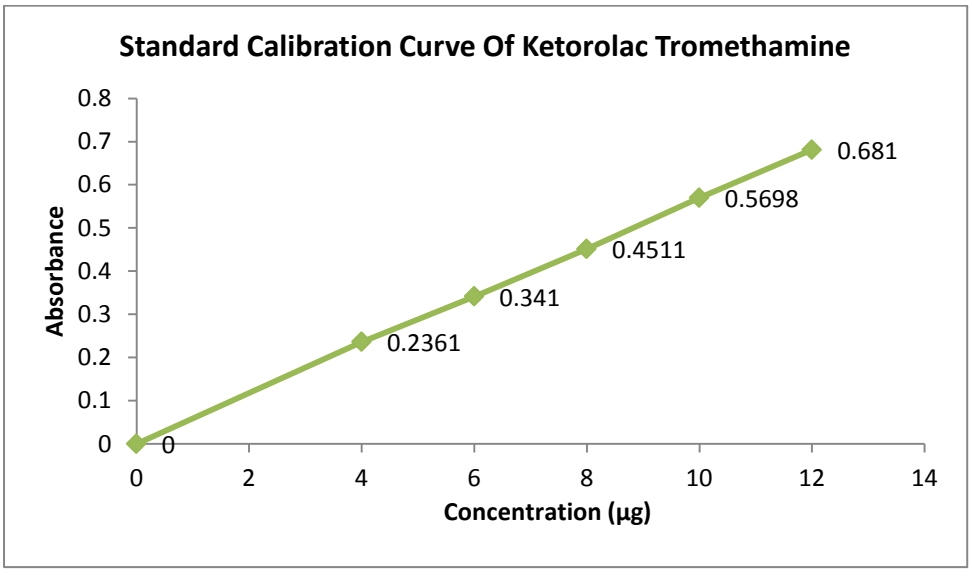

Figure 7: Standard calibration Curve of Ketorolac Tromethamine

***Ex vivo* Transcorneal permeation studies**

Table 5: Release rate and percentage permeability of Ketorolac Tromethamine from the formulations

Product Code	Vol. of formulation placed over the cornea at donor cell (ml)	Conc. over the cornea at donor cell (µg/ml)	Vol. of receptor cell (ml)	Absorbance of 1ml test solution collected after 120 min	Drug conc. at receptor cell after 120 min (µg/10ml)	Conc. at receptor cell (µg/ml)	Corneal percentage (%/ml)
KT	1	1000	10	0.0491	4.9	0.49	0.49
KTVC	1	1000	10	0.412	71.2	7.12	7.12
KTPC	1	1000	10	0.464	81.6	8.16	8.16
KTLC	1	1000	10	0.5971	104.9	10.49	10.49

KT : Ketorolac Tromethamine. KTVC : Ketorolac Tromethamine and Valine conjugated mixture. KTPC : Ketorolac Tromethamine and Phenyl alanine conjugated mixture. KTLC : Ketorolac Tromethamine and Valine conjugated mixture. KTVC : Ketorolac Tromethamine and Leucine conjugated mixture.

(Mean Value of Absorbance *n=3)

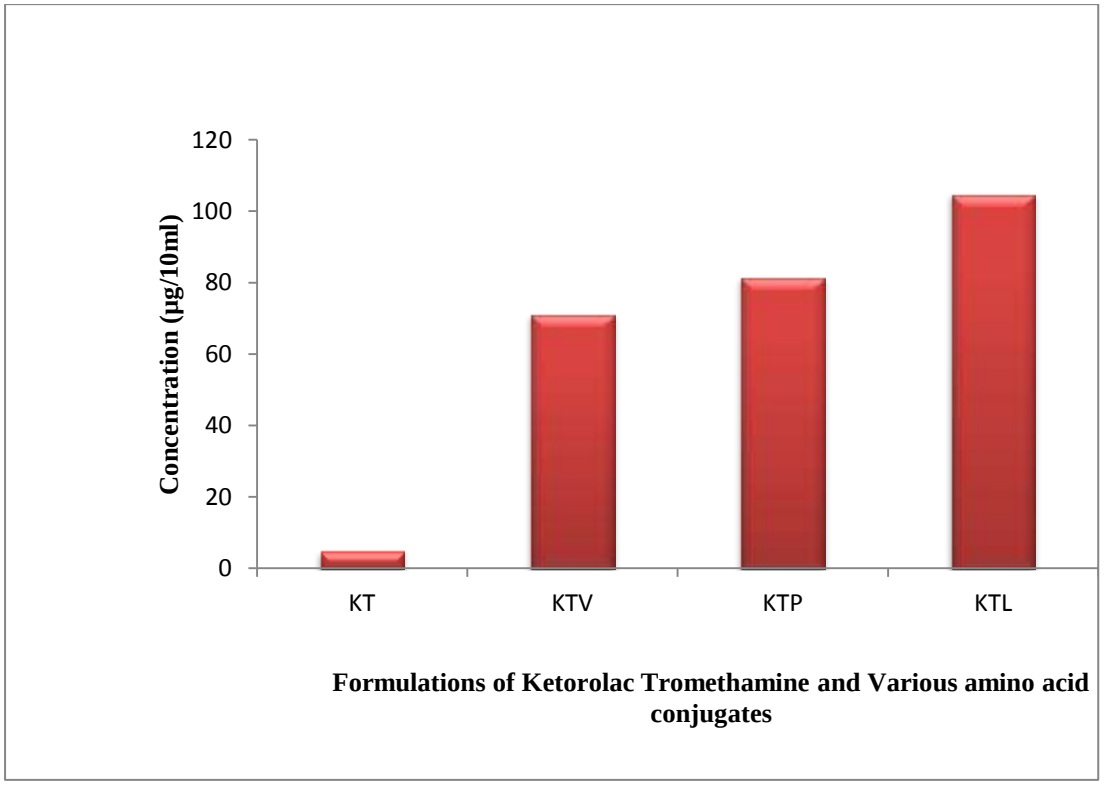

Figure 8: Release rate of standard and test formulations

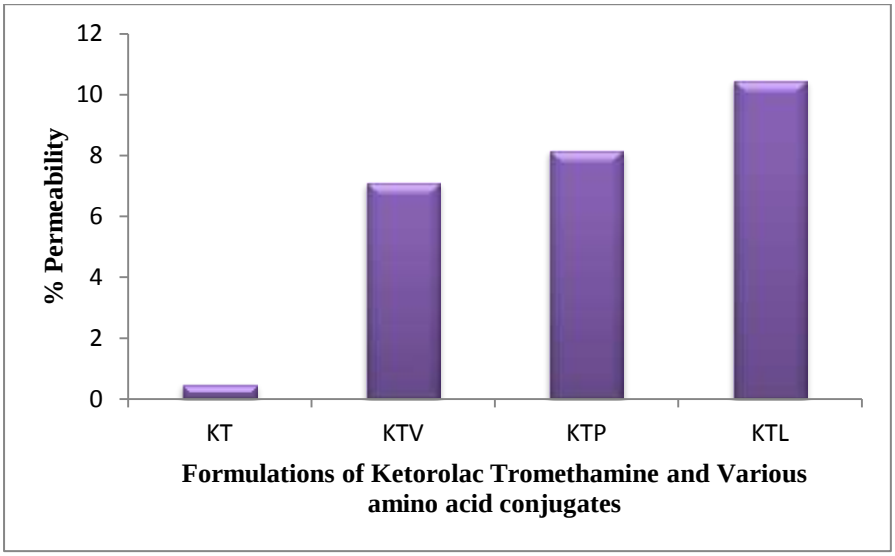

Figure 9: Percentage permeation of Ketorolac tromethamine from formulations

Percentage Cornea hydration of formulations

Table 6: The percentage of corneal hydration of Ketorolac Tromethamine formulations

Product Code	Weight of dried cornea (gm)	Weight of wet cornea (gm)	Percentage of Corneal Hydration (120 minutes) (%)
KT	0.423	0.7581	76.11
KTL	0.3641	0.8016	79.33
KTP	0.381	0.7691	80.48
KTV	0.3882	0.7894	77.5

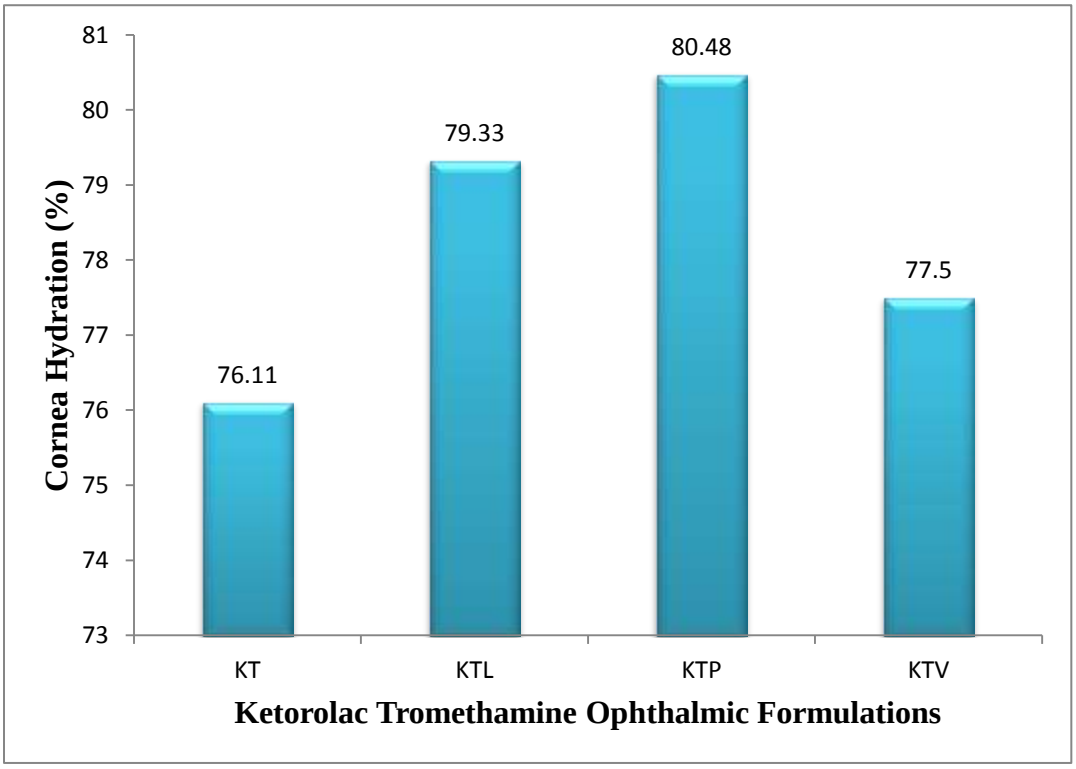

Figure 10: Percentage Corneal hydration of formulations

Summary of percentage of Ketorolac Tromethamine permeation and cornea hydration analysis

Table 7: Summary of percentage of Ketorolac Tromethamine permeation and cornea hydration analysis

Test solution	Amount Permeated (mg) (120 minutes)	Permeation (%) (120 minutes)	Corneal Hydration (%)
Ketorolac Tromethamine (0.5% w/v)	0.00049±0.000029	0.49±0.029	76.11
Ketorolac Tromethamine and Lysine conjugate	0.0105±0.000072	10.49±0.072	79.33
Ketorolac Tromethamine and Phenylalanine conjugate	0.0081±0.00021	8.16±0.21	80.48
Ketorolac Tromethamine and Valine conjugate	0.0071± 0.00017	7.12±0.17	77.5

(Values are mean ± SD of 3 samples from each group)

In the study a standard ophthalmic solution of Ketorolac Tromethamine was prepared with suitable excipients as shown in Table.1 and three test formulations of Ketorolac Tromethamine conjugated with three different amino acid transporters such as Lysine, Phenylalanine and Valine were formulated by using the ingredients mentioned in Table.2. The process of conjugation was carried out by using solvent evaporation technique in a rotary evaporator by using the quantities of Ketorolac Tromethamine with amino acids as mentioned in Table.3. The prepared conjugates were observed to possess good physical characterization[10] with the respect to colour, odour and nature in which white to off white crystalline substances were formed with odourless nature and the observations were shown in Table.4. The prepared conjugates were subjected to thin layer chromatography in order to check their purity and the results obtained for Conjugates of Ketorolac Tromethamine and Valine were shown in Figure:1. All the formulations were subjected to ex-vivo transcorneal permeation studies on excised goat cornea by using all glass Franz diffusion cell. The isolated fresh eye ball of goat pinned on a dissection board was shown in Figure.1.and the excised cornea along with 2-4mm of sclera tissue was shown in Figure.2. The excised cornea was mounted in between the donor and the receptor compartments of the cell and the picture was shown in Figure.3.The receptor compartment of the cell was filled with bicarbonate ringer solution with a Teflon coated magnetic bead as shown in the Figure.4. The cell was placed in a beaker containing distilled water which was maintained with a temperature of 37^0 C on a hot plate as shown in Figure.5. To the donar compartment 1 ml samples of the prepared formulations were introduced separately with a fresh excised cornea mounted in the cell for each and the samples from the receptor compartment were collected by replacing with equal amounts of bicarbonate ringer

solution and subjected for UV analysis at 322nm. Observations of Standard Calibration curve Ketorolac Tromethamine were obtained from UV spectrophotometry by measuring the absorbance of standard solution at 322nm and the calibration curve was shown in Figure.7. The results of release rate and percentage permeability of Ketorolac Tromethamine from the prepared formulations were mentioned in Table.5. The results on comparison revealed that the formulations of the drug with amino acids exhibit higher release rate when compared with standard formulation. The comparative plots of same were shown in Figure 8 and 9 respectively. The percentage corneal hydration was calculated for each formulation and all the results were observed to be in the standard range and were tabulated in Table.6 and the same were graphically represented in Figure.10. All results were summarized in Table.7 and the release rate of all the 3 formulation of Ketorolac Tromethamine and amino acid conjugates (KTP, KTV, and KTL) were found to be rapid when compared with standard formulation of Ketorolac Tromethamine as shown in Figure.8. Conjugates of Ketorolac Tromethamine with Lysine have shown the maximum release rate among all the other formulation. The percentage of permeability of the formulations were calculated based on the concentration permeated through the excised cornea were illustrated in Figure.9. Based on the results of release rate and percentage permeation studies done, it was evidential that the formulations of Ketorolac Tromethamine with amino acids in the form of conjugates has rapid release rate and increased corneal permeation of Ketorolac. Another factor being considered in this formulation is cornea hydration. The level of corneal hydration is an important indicator of corneal irritation and therefore percentage corneal hydration. Percentage of corneal hydration of Ketorolac Tomethamine and various amino acid conjugates were found to remain in normal range. This indicates all the formulation prepared were not causing any damage to cornea.

CONCLUSION

Based on the results, the study have concluded that the drug Ketorolac Tromethamine conjugated with amino acid transporters were efficient to enhance the transcorneal permeation of Ketorolac. The study also revealed that ophthalmic formulation of Ketorolac Tromethamine conjugated with amino acids have gain maximum ocular bioavailability through excised goat's cornea at the posterior segment. The results also conclude that the Ketorolac Tromethamine conjugated with amino acid transporters does not cause any damage or irritation to the cornea. Among all the formulations, Ketorolac Tromethamine conjugated with Lysine was found to exhibit the best outcome in terms of percentage of drug release and percentage of cornea permeation comparatively. Therefore, the amino acid Lysine can be concluded as best suitable amino acid

transporter for Ketorolac Tromethamine ophthalmic solution which can provide maximum bioavailability at the posterior segment of eye as an ophthalmic targeted drug delivery.

REFERENCES

1. Boddu SHS, Nesamony J. Utility of transporter/receptor(s) in drug delivery to the eye. World J Pharmacol 2013; 2: 1-17.
2. Vivek D, Paliwal S. A novel approach to formulation factor of aceclofenac eye drops efficiency evaluation based on physicochemical characteristic of in vitro and in vivo permeation, Saudi pharmaceutical J 2013; 22: 240-245.
3. Koichi B,Yuji T,Akira K, Hitoshi K, Shunji Y, Hachiro N, Kohji N. A method for enhancing the ocular penetration of eye drops using nanoparticles of hydrolyzable dye, J of Controlled Release 2011; 278-287.
4. Gu S, Roderick HL, Camacho P, Jiang JX. Characterization of an N-system amino acid transporter expressed in retina and its involvement in glutamine transport, J Biol Chem 2001; 276: 24137-24144.
5. Sharma A, Pathak M, Sharma D, Ahmad S. Enhancement of transcorneal permeation of Diclofenac using various amino acids as permeation enhancers, Novel Science International Journal Of Pharmaceutical Sci 2012; 1(5): 267-273.
6. Munish A, Avinash S, Surendra K, Dipak K. Topical Ocular Delivery Of NSAIDs, AAPS J 2008; 10: 229-241.
7. Plummer D.T, An introduction to Practical biochemistry, Tata McGraw – Hill Pow DV. Amino acids and their transporters in the retina, Neurochem. Int 2001; 38: 463-484.
8. Bhople A, Deshpande S, Sheiakh S, Patange H, Chandewar A. Formulation and Development of Ketorolac Tromethmaine Ophthalmic Solution. J Bioequiv 2013; 5: 099-109.
9. Fegade JD, Mehta HP, Rajesh Y, Chaudhari, Patil VR. (2009) Simultaneous Spectrophotometric Estimation of Ofloxacin and Ketorolac Tromethamine in Ophthalmic Dosage Form. Int J ChemTech Res 2009; 2: 189-194.
10. Gandhi MD, Pal D, Mitra AK. Identification and functional characterization of a Na(+)-independent large neutral amino acid transporter (LAT2) on ARPE-19 cells, Int J Pharm 2004; 275; 189-200.
11. Katragadda S, Zhu X, Talluri SR, Ashim KM.Small Neutral Amino Acid Ester Prodrugs Of Acyclovir Targeting Amino Acid Transporters On The Cornea: Possible Antiviral Agents Against Ocular Hsv-1 Infections, Ophthalmol Eye Disease 2010; 2: 43-56.