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Effect of Cosolvents on the Absorptive Clearance of Ketotifen Fumarate from Rabbit Intestine, *In-situ*

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ABSTRACT

Objective: Investigate the effect of ethanol, polyethylene glycol 400, propylene glycol, glycerol and sorbitol on the absorptive clearance of ketotifen fumarate in the rabbit. **Methods:** *In-situ* intestinal perfusion technique, "through and through" was used for estimation of membrane transport parameters of ketotifen fumarate from duodenum, jejunum, ileum and ascending colon in the rabbit. These parameters include absorptive clearance per unit length PeA/L (ml/min.cm), percentage fraction absorbed per unit length (% Fa/cm) and anatomical length that required for complete absorption in specific segment (L95%). **Results:** The absorption was in the order ascending colon> duodenum > jejunum> ileum; where the absorptive clearance normalized to intestinal segment length PeA/L (ml/min.cm) was 0.0071 ± 0.0003, 0.0058 ± 0.0001, 0.0051 ± 0.0001, and 0.0047 ± 0.0001 in each segment respectively. The effect of cosolvents in jejunum was in the order; ethanol 15% >glycerol 30% > propylene glycol (PG40%) > polyethylene glycol400 (PEG-400 40%) >sorbitol 40%, Where the absorptive clearance normalized to intestinal segment length PeA/L (ml/min.cm), mean ± SE was: 0.0142 ±0.0011, 0.0086 ± 0.0002, 0.0075 ± 0.0003, 0.0022 ± 0.0001, and 0.0014 ± 0.0001 for each cosolvents respectively. The same order was obtained in the ascending colon. **Conclusion:** The enhancing action of the ethanol, propylene glycol and glycerol may be due fluidization of the cell membrane with a subsequent increase in transcellular absorption, while the inhibitory effect of polyethylene glycol and sorbitol could attributed to water secretion, H-bonding formation and reduced thermodynamic activity of drug molecules.

Keywords: *Cosolvents; Intestinal absorption; Intestinal perfusion; In situ intestinal absorption; Ketotifen absorption; Ketotifen bioavailability*

INTRODUCTION

Ketotifen fumarate is a selective, second-generation non-competitive histamine antagonist (H_1-receptor) and mast cell stabilizer[1]. Ketotifen inhibits the release of mediators from mast cells involved in hypersensitivity reactions. It is available in two forms: ophthalmic form to treat allergic conjunctivitis, and in oral form to prevent asthma attacks[2]. Ketotifen is almost completely absorbed from the gastrointestinal tract following oral administration; its oral bioavailability is reported to be only about 50 % due to hepatic first-pass metabolism. Ketotifen N-glucuronide was reported to be the major metabolite, which is excreted in urine and feces as 50 % of dose[3,4].

Ketotifen fumarate is available in tablet, syrup, and drops for oral administration in adults, children, and infants. Since ketotifen fumarate is sparingly soluble in water (15.3 mg/L) [5-7], Cosolvents are frequently used to improve its aqueous solubility in liquid dosage forms. The primary objective of this research was to investigate the influence of cosolvents such as ethanol, glycerol, propylene glycol (PG), polyethylene glycol 400 (PEG-400) and sorbitol on the absorptive clearance of ketotifen fumarate from rabbit intestine, adopting in-situ intestinal perfusion technique. The second objective

was to investigate qualitative and quantitative transport parameters of ketotifen fumarate across the intestinal membrane. This could be of significant importance in design and development of liquid oral dosage forms, and optimizing the bioavailability of this drug. The suitability of the rabbit as an animal model for gastrointestinal drug absorption studies was reported[8-10].

MATERIALS AND METHODS

Material

Ketotifen fumarate and ethyl paraben were obtained from Sigma Chemical Co., St Louis, Mo, USA. Acetonitrile, methanol (HPLC grade), ethanol, glycerol, and sorbitol were obtained from BDH laboratory, England. Polyethylene glycol 400 (PEG-400), propylene glycol (PG), phosphoric, sodium dihydrogen phosphate, sodium chloride 0.9% for injection were obtained from El Nasr Pharmaceutical Chemicals Company, Cairo, Egypt. Ketamine HCl (100 mg/ml) was obtained From Sigma Pharmaceutical Company, Quesina, Egypt. Chlorpromazine HCl (25 mg/ml) was obtained From Misr Pharmaceutical Company, Cairo, Egypt. Double distilled water for HPLC analysis was used. Membrane filters 0.45 µm was obtained from Millipore, Milford, U.S.A.). All chemicals were of analytical grade.

Equipment

Constant rate perfusion pump (Harvard-22, Harvard Apparatus, Millis, MA, USA), Timer (Brannan, England), PH meter (HANNA Instruments, Romania), Sonicator (ultrasonic 57X, Clean America, N.Y., USA). Water bath (KOWELL N4, Germany), Centrifuge (Minor 35, M.S.D., L.T.D., England). HPLC (WatersTM 600 controller, USA) equipped with a variable wavelength detector (Waters TM 486, Tunable Absorbance Detector, USA) and an automatic sampling system (Waters TM 717 Plus Autosampler, USA) and the whole equipment is under computer control. Reversed-phase column 15 cm X 3.9 mm (i.d.) C18, µ Bondapak™, Waters, with an average particle size of 5 µm.

Preparation of the drug solutions for intestinal perfusion

The study protocol and animal manipulations were conducted as per the approval of the Ethical Committee of College of Pharmacy, Tanta University (Approval number, 16012016). Rabbits were obtained from animal house, collage of agriculture Kafr El-Sheikh University, Egypt. The animals were housed in the animal house, department of pharmacology, collage of pharmacy until the day of experiment, the housing was at room temperature and the animal was allowed for food and water. The animal was fasted over night before the day of the experiment but allowed for water. Perfusion solution containing 0.5 µg/ml (0.001mM) ketotifen fumarate was prepared by weighing the exact amount of ketotifen fumarate and dissolving in 0.9% (w/v) sodium chloride to obtain the required concentration.

Segment preparation

The procedures for preparation of the isolated intestinal segments for the *in-situ* perfusion experiment were described in details previously[11-13]. On the day of the study the rabbit was anesthetized using chloropromazine HCl injection (2 mg/kg) as muscle relaxant with ketamine HCl being used as the main anesthetic agent. Ketamine HCl was was delivered in two successive doses (45 mg/kg) at 15 minutes intervals. Smaller dose of the anesthetic (25 mg/kg) was injected if required. The anesthetized rabbit is mounted on a thermostated matrix with the animal being in a supine position. Abdominal hair was removed, the skin was cleaned before making a 6-8 cm midline abdominal incision. The target intestinal segment was exposed and the required length was cannulated from both sides after tying with surgical thread. The cannulated segment was cleaned using warm saline. The measured length depended on the target segment with length being 15, 30, 30, and 10 cm for duodenum, jejunum, ileum and ascending colon, respectively.

Experimental design

Seven groups each consisted of three male, albino rabbits weighing 2.8-3.1 kg were used in these experiments. The first group was utilized to estimate the membrane transport parameters of ketotifen fumarate in both the jejunum and ascending colon of the rabbit intestine. The second group was utilized to estimate the same parameters in the duodenum and ileum. The other five groups were utilized to examine the effect of ethanol (15 %), polyethylene glycol400 (PEG-400 40%), propylene glycol (PG 40%), glycerol (30 %) and sorbitol (40%) on the absorptive clearance of ketotifen fumarate from both the jejunum and ascending colon. The drug concentration was 0.5µg/ml (0.001 mM), which was perfused either alone or with a cosolvent (n=3).

In Situ intestinal perfusion

Solutions containing ketotifen fumarate (0.001 mM) in normal saline were perfused at a flow rate of 0.27 ml/min. The intestinal effluent samples were collected at 10 minutes intervals for 120 minutes in 10 ml pre-weighed stoppered tubes. These tubes were weighed again after sample collection, and the effluent weight was recorded as the difference. Intestinal net water flux was estimated gravimetrically.

Table 1. Membrane transport parameters of ketotifen fumarate in four different segments of rabbit intestine

Parameter	Duodenum	Jejunum	Ileum	Ascending colon
Segment length (cm)	15	30	30	10
PeA	0.0809 (0.0071)	0.1421 (0.0051)	0.1401 (0.0042)	0.0954 (0.0107)
Rout/Rin	0.7259 (0.0287)	0.5696 (0.0141)	0.5621 (0.0300)	0.6874 (0.0369)
PeA/L (ml/min.cm)	0.0058 (0.0001)	0.0051 (0.0001)	0.0047 (0.0001)	0.0071 (0.0003)
%Fa/cm	1.9504 (0.0929)	1.5584 (0.0518)	1.4596 (0.0999)	2.3309 (0.1276)
l*(L95%) (cm)	156.553 (24.288)	154.5784 (0.7677)	164.2637 (9.7057)	118.0405 (9.3394)
ARL (cm)	-96.5532 (26.07)	-34.5784 (22.71)	-104.2637 (44.53)	-103.0405 (44.14)
JW (ml/min)	0.0385 (0.0180)	0.0375 (0.0146)	0.0344 (0.0186)	0.0342 (0.0144)
JW/L (ml/min.cm)	0.0027 (0.0011)	0.0014 (0.0005)	0.0011 (0.0006)	0.0025 (0.0009)

PeA is the overall absorptive clearance, Rout/Rin is the fraction remaining to be absorbed, %Fa is the percentage fraction absorbed, PeA/L is the effective permeability surface area product normalized to the segment length, % Fa/cm is the percentage fraction absorbed per unit length, L95% is the length required for 95% absorption, ARLs is the anatomical reserve length which is the difference between the total length of the total anatomical segment and the L95%, JW is the water flux and JW/L is the water flux normalized to the segment length. Values between brackets are ± standard error, n = 3.

Table 2. Average Lag time normalized to intestinal length for ketotifen fumarate in four different anatomical sites

Intestinal segment	Average lag time (min)	Average length (cm)	Average Lag time/length (min/cm)
Ascending colon	14 *(0.62)	10 *(0.27)	1.4
Duodenum	11.5 (0.33)	14.5 (0.25)	0.79
Jejunum	13 (1.17)	29.2 (0.89)	0.44
Ileum	12 (0.145)	29.4 (0.60)	0.4

**Values between brackets are ± standard error, n = 3*.

Table 3. Effect of water flux (ml/min.cm) on the diffusive and convective absorptive clearances of ketotifen fumarate in four different anatomical sites of rabbit intestine

Regression parameters	Duodenum	Jejunum	Ileum	Ascending colon
Intercept (DAKp/Δx)	0.0051* (0.00075)	0.0043* (0.0002)	0.0045* (0.0002)	0.0056* (0.0006)
Slope (∅)	0.257*** (0.29)	0.589* (0.129)	0.129*** (0.135)	0.593* (0.166)
Coefficient of determination (R^2)	0.0694	0.565	0.0544	0.4425

Values in brackets are ± standard error values, n=3. (DAKp/Δx) is the permeability coefficient and (∅s) is the sieving coefficient.
** Statistically different from zero (P<0.05).*
****Statistically not different from zero (P>0.05).*

Chromatography

Standard solutions

A 6-point standard curve was prepared by adding appropriate volumes of ketotifen fumarate stock solution in methanol into a series of 13- ml ground glass stoppered centrifuge tubes in the amount of 1, 2, 4, 6, 8 and 10 µg. Each of these tubes was spiked with 50 µg of ethyl paraben as an internal standard. The methanol was evaporated off and the residue was reconstituted in one ml of the mobile phase, vortex mixed, and 100 µl were transferred to micro vials which were loaded on automatic sampler after crimping for injection.

Sample analysis

The concentration of ketotifen in the perfusate samples was quantified using the developed HPLC method. This involved centrifugation of the perfusate samples and loading of known volume of the perfusate sample into tubes previously spiked with the specified amount of the internal standard (50 µg). This was subjected to vortex mixing before loading into the HPLC vials, **Figure1**.

Data analysis

Absorptive Clearance

The concentration of Ketotifen fumarate recorded in each sample was corrected with respect to the net water flux to produce the actual amount of drug remaining in each sample (C_{out}). The ratio between C_{out} and the amount of drug perfused C_{in} was used to calculate the fraction of drug remaining after perfusion. The fraction remaining at steady state $\{(C_{out} /C_{in})ss\}$ was taken from the average of the fractions remaining in samples collected during the second hour of perfusion. This was used to calculate the absorptive clearance (PeA) expressed as ml/minute using the following equation[14-18], in which A, represents the effective surface area (cm^2), Pe denotes the apparent permeability coefficient (cm/min) and Q is the average flow rate of the perfusate through the target segment (ml/min).

$$\text{“}PeA = -Q * \ln \{C_{(out)} /C_{(in)}\}_{ss}\text{”} \quad (1)$$

The fraction of the drug absorbed at steady state (Fa) was calculated using the following equation.

$$\text{“}Fa = 1- \{C_{(out)} /C_{(in)}\}_{ss} = 1- \exp^{-(PeA/Q)}\text{”} \quad (2)$$

The length of the intestine remaining after complete drug absorption is described as the anatomical reserve length (ARL) and was calculated from the following equation in which L* is the anatomical length of given intestinal segment and l* is the intestinal length along which complete absorption is achieved. These lengths were expressed in centimeters.

$$\text{“}ARL = (L^*) - (l^*)\text{”} \quad (3)$$

Practically, it is difficult for the solute to disappear completely form the intestinal lumen especially in a logarithmic situation. Accordingly, a 5% fraction was assumed to remain with 95% absorption being taken as an approximate reflection of complete absorption[18]. Taking this into consideration the length required for 95% absorption (L95%) was calculated using the following equation.

$$\text{“}0.05 = \exp^{-\{(PeA.\, l^*)/Q\}}\text{”} \quad (4)$$

Where PeA is absorptive clearance normalized to length and l* is L95% for the given drug.

Effect of Solvent drag on intestinal absorptive clearance:

To investigate the pathway of drug transport across the intestinal membrane it was essential to monitor the drug absorption as a function of water flux. This was achieved by plotting the absorptive clearance as a function of net water flux Jw (ml/min) which was calculated from the difference between the volume of the perfusate pumped during a given time period (Q_{in}) and the volume of perfusatere covered from the segment during this time (Q_{out}). This is simplified by the following equation.

$$\text{“}J_w = Q_{in}\text{-}Q_{out}\text{”} \quad (5)$$

The rate of drug absorption (Js) which is calculated in µg/min depends on the contribution of diffusive and convective processes. The net amount of drug absorbed per unit time can by driven from the following equation is then given by:[19-21]

$$\text{“}J_s = K_s (C\text{-}C_p) + Ø_s J_w\, C\text{”} \quad (6)$$

Where the diffusive process is described by $[K_s (C\text{-}C_p)]$ in which K_s is the diffusive permeability coefficient of the drug. The concentration of the drug in the intestinal lumen is expressed as C with that in the plasma being defined as C_p. and the convective process is represented by $[Ø_s J_w\, C]$ in which $Ø_s$ is the sieving coefficient of drug. Equation (6) can be approximated to equation (7) at steady state, taking into consideration the existence of sink conditions in blood.

$$\text{“}J_{ss} = DAKp /\Delta x\, (C_{ss}) + Ø_s J_w\, (C_{ss})\text{”} \quad (7)$$

Where $J_{ss}$is the steady state solute flux (µg/min), D is the diffusion coefficient of the drug, A is the effective surface area of drug absorption Kp is the (octanol / water) partition coefficient of the compound. Δx is the path length. C_{ss} is the length averaged steady state concentration of the solute in the lumen (µg/ml). Rearrangement of equation (7) gives:

$$\text{“}J_{ss}/C_{ss} = DAK_p/\Delta x + Ø_s J_w\text{”} \quad (8)$$

The term J_{ss}/C_{ss} represents the overall absorptive clearance of the given solute (ml/min) which is achieved via different permeation pathways and can be practically calculated as permeability surface area product, PeA.

Statistical analysis

The membrane transport parameters of ketotifen fumarate were determined in duodenum,

Table 4. Effect of ethanol, propylene glycol (PG), and glycerol on membrane transport parameters of ketotifen fumarate in jejunum of rabbit intestine

*Parameter	Control	Ethanol 15%	Glycerol 30%	PG 40%
Segment length (cm)	30	30	30	30
PeA	0.1421 **(0.0051)	0.3647 (0.0241)	0.2505 (0.0154)	0.2077 (0.0166)
Rout/Rin	0.5696 (0.0141)	0.2380 (0.0228)	0.3693 (0.0265)	0.4359 (0.0292)
PeA/L (ml/min.cm)	0.0051 (0.0001)	0.0142 (0.0011)	0.0086 (0.0002)	0.0075 (0.0003)
% Fa/cm	1.5584 (0.0518)	2.9710 (0.1132)	2.1739 (0.0222)	2.0383 (0.0133)
l*(L95%) (cm)	154.5784 (0.7677)	53.9056 (4.0506)	87.7412 (3.6370)	100.8589 (3.8610)
ARL (cm)	-34.5784 (22.71)	66.0944 (19.01)	32.2588 (7.51)	19.1411 (5.47)
JW (ml/min)	0.0375 (0.0146)	0.0369 (0.0089)	0.0393 (0.0105)	0.0457 (0.0008)
JW/L (ml/min.cm)	0.0014 (0.0005)	0.0014 (0.0003)	0.0013 (0.0003)	0.0017 (0.0001)

Parameters are the same of **Table-1*
***Values between brackets are ± standard error, n = 3.*

Table 5. Effect of ethanol, propylene glycol (PG), and glycerol on membrane transport parameters of ketotifen fumarate in ascending colon of rabbit intestine

*Parameter	Control	Ethanol 15%	Glycerol 30%	P.G 40%
Segment length (cm)	15	15	15	15
PeA	0.0954 **(0.0107)	0.2074 (0.0234)	0.1754 (0.0238)	0.1103 (0.0014)
Rout/Rin	0.6874 (0.0369)	0.4792 (0.0381)	0.5020 (0.0445)	0.6467 (0.0054)
PeA/L (ml/min.cm)	0.0071 (0.0003)	0.0188 (0.0007)	0.0125 (0.0014)	0.0092 (0.0001)
Fa/cm	2.3309 (0.1276)	4.7507 (0.1392)	3.5719 (0.2776)	2.9443 (0.0447)
l* (L95%) (cm)	118.0405 (9.3394)	44.8989 (1.6237)	62.5757 (6.6170)	88.8510 (3.0592)
ARL (cm)	-103.0405	-29.8989	-47.5757	-73.851
JW (ml/min)	0.0342 (0.0144)	0.0176 (0.0015)	0.0400 (0.0021)	0.0425 (0.0066)
JW/L (ml/min.cm)	0.0025 (0.0009)	0.006 (0.0000)	0.0003 (0.0001)	0.0035 (0.0005)

Parameters are the same of **Table-1*
***Values between brackets are ± standard error, n = 3.*

jejunum, ileum and ascending colon of the rabbit intestine when Ketotifen fumarate was perfused alone or in the presence of each cosolvent. The unpaired T-Test was utilized to compare pharmacokinetic parameters obtained. P-value <0.05 was considered significant.

RESULTS AND DISCUSSION

Regional differences in the absorption of ketotifen fumarate from rabbit intestine:

Ketotifen fumarate was incompletely absorbed from the rabbit small and large intestine. The membrane transport parameters of this compound were estimated

and summarized in **Table 1**. The absorptive clearance normalized for the intestinal length in each segment (PeA/L) indicate that the absorption of ketotifen fumarate significantly predominates (P-value < 0.05) in the ascending colon as compared to duodenum, jejunum, and ileum.

The calculated values for absorptive clearance normalized to the intestinal length (mean ± S.E.) were:0.0071 ± 0.0003, 0.0058 ± 0.0001, 0.0051 ± 0.0001, and 0.0047 ± 0.0001 ml/min.cm in ascending colon, duodenum, jejunum, and ileum respectively.

These results indicate that the absorption of ketotifen fumarate was in the order: ascending colon>duodenum>jejunum>ileum, **Figure 2**. The major reason for this order is the fact that Ketotifen fumarate is a substrate for p-glycoprotein [6]. Since the amount of p-glycoprotein increase distally from the duodenum to the ileum over approximately 10 fold range, While in the large intestine, the absolute amount is much less than that present in the small intestine [22,23]. So the least absorption of ketotifen fumarate was from the ileum.

The experimental results indicate that the lag time normalized to intestinal lengths for ketotifen fumarate was: 1.4, 0.79, 0.44 and 0.4 min/cm in ascending colon, duodenum, jejunum, and ileum respectively (**Table 2)**. This means that the mean residence time was in the order: ascending colon > duodenum > jejunum > ileum, which was reflected as the same order of absorptive clearance.

The percent fraction absorbed per cm (% Fa/cm) at steady state for ketotifen fumarate was (2.3309 ± 0.1276), (1.9504 ± 0.0929), (1.5584 ± 0.0518), and (1.4596 ± 0.0999) in ascending colon, duodenum, jejunum, and ileum, respectively.

The length required for complete absorption of ketotifen fumarate (L95%) reached (118.0405 ±9.3394 cm), (156.5532 ± 24.2886 cm), (154.5784 ± 0.7677 cm), and (164.2637 ± 9.7057 cm), in ascending colon, duodenum, jejunum, and ileum, respectively. These values indicate that the absorption of ketotifen fumarate requires intestinal length longer than that of the actual anatomical length of rabbit intestine so that the anatomical reserve length values (ARL) were negative as indicated in **Table 1**.

These negative values reflect the incomplete absorption of this compound from the entire length of the rabbit intestine with a net result of low bioavailability of this compound which reaches only 50%. So that, the poor transport across the intestinal membrane together with pre-systemic metabolism in the intestinal lumen and liver may account for the reduced overall bioavailability of ketotifen fumarate [6,24,25].

Effect of the estimated solvent drag on absorptive clearance of ketotifen fumarate

The effect of net water flux on the absorptive clearance of ketotifen fumarate was studied according to Lifson's model[19,20]. Plots of ketotifen fumarate absorptive clearance versus the net water flux both normalized to the segment length at steady state are presented in **Figure 2**. for duodenum, jejunum, ileum and ascending colon respectively, (n= 3). Equation (8) was fitted to data by linear regression and the regression parameters for the different intestinal segments are depicted in the **Table 3**.

For duodenum, jejunum, ileum and ascending colon; the intercepts obtained from the linear regression equation were:0.0051, 0.0043, 0.0045 and 0.0056, respectively (statistically different from zero p-value < 0.05). This means that the transcellular diffusive pathway contributed to 87.9 %, 84.3 %, 95.7 % and 78.9 % of the overall absorptive clearance in the four segments respectively. The paracellular convective pathway contributed to 12.1 %, 15.7 %, 4.3% and 21.1% of the overall absorptive clearance of ketotifen fumarate from duodenum, jejunum, ileum and ascending colon. However, these values were not statically different from zero where the slopes obtained from linear regression equations were not significantly different from zero in all the four segments studied (p-value > 0.05).

Thus, results show that ketotifen fumarate is mainly transported by the transcellular diffusive pathway in the four segments; while the paracellular convective pathway had a minor role in its absorption. This could be attributed to the lipophilic nature of ketotifen fumarate (log P value of 2.2) and poor aqueous solubility [6-7].

Effect of cosolvents on Membrane transport parameters of ketotifen fumarate from rabbit intestine

Enhancing effect of ethanol, glycerol, and propylene glycol (PG)

The effect of cosolvents (ethanol, glycerol, PG) on membrane transport parameters of ketotifen fumarate from jejunum and ascending colon of rabbit intestine are depicted in **Figure 3**, and **Tables 4** and **5**. Ethanol, glycerol, and PG had significantly increased the absorptive clearance (PeA/L) and the percentage fraction absorbed per unit length (%Fa/cm) of ketotifen fumarate from jejunum and ascending colon (P-value < 0.05). The enhancing action of the cosolvents on membrane transport of ketotifen fumarate was in the order of ethanol 15% >glycerol 30% > PG40%.

The percent increase in the (PeA/L) of ketotifen fumarate in jejunum and ascending colon after addition of 15% ethanol was almost 100% in both segments respectively. Glycerol had increased the

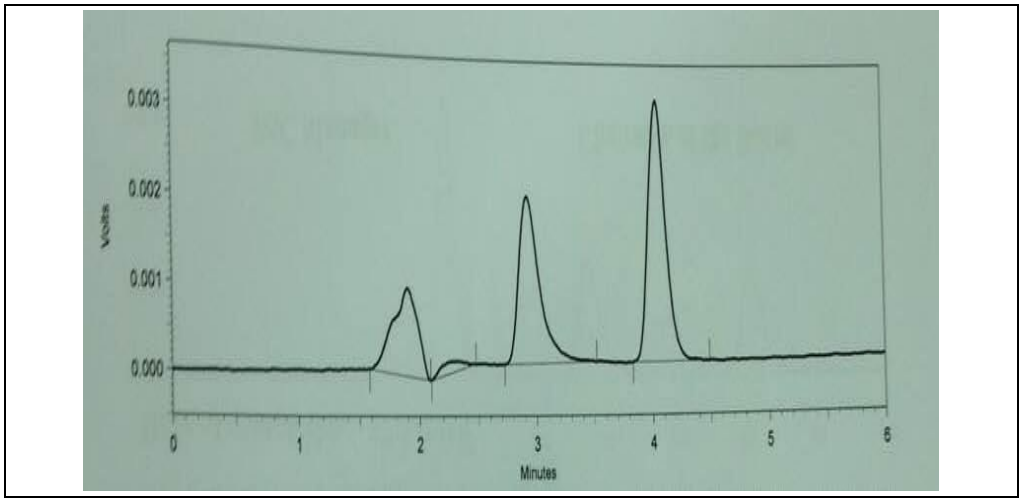


Figure 1. Typical chromatogram obtained during analysis of ketotifen fumarate perfusate sample showing the retention time of the drug at 3.113 min and ethyl paraben (I.S) at 4 min.

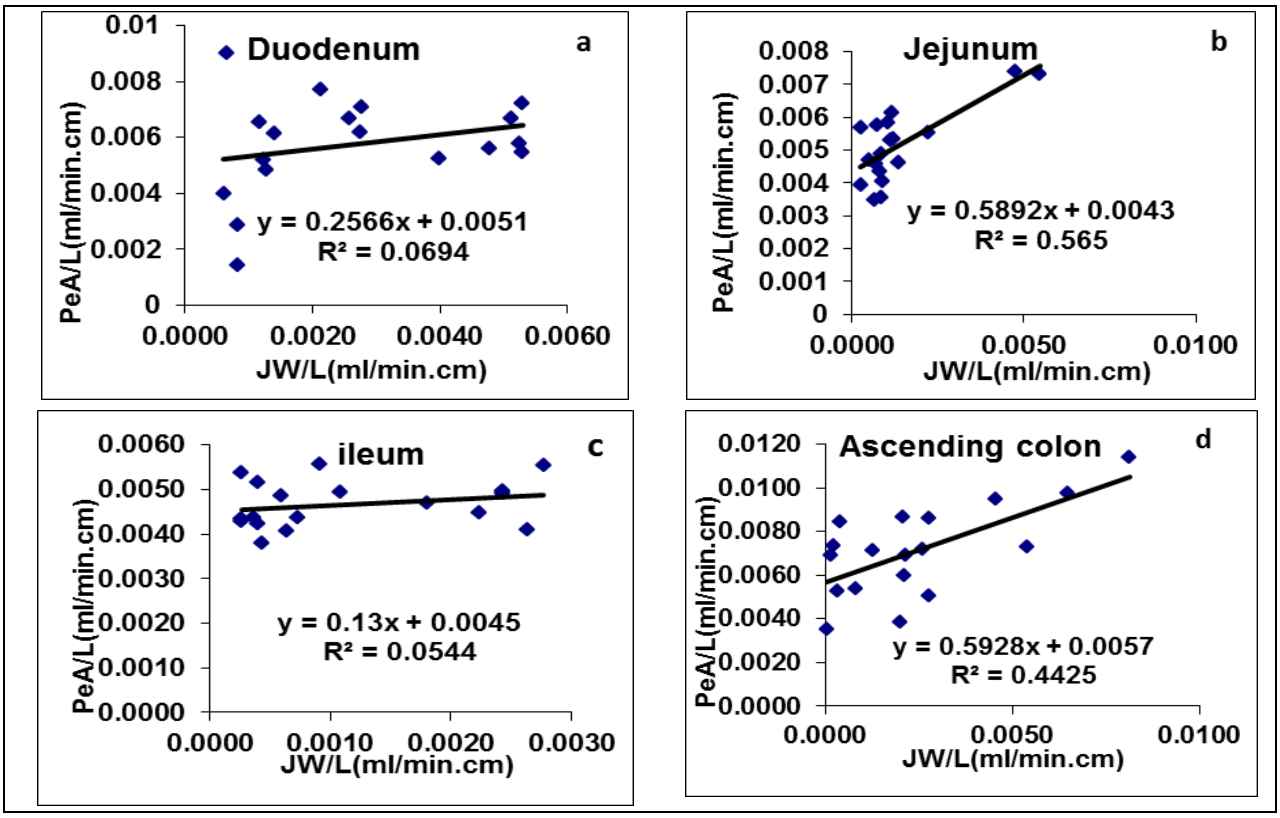


Figure 2. Absorptive clearance of ketotifen fumarate versus water flux in investigated intestinal segments: (a) duodenum, (b) jejunum, (c) ileum and (d) ascending colon. Parameters are normalized to segment length. Lines represent least square linear regression of data points

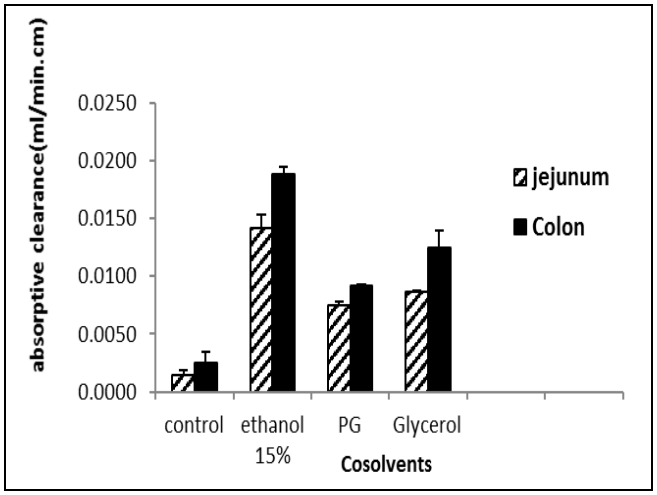


Figure 3. Effect of ethanol, PG, and glycerol on the absorptive clearance of ketotifen fumarate from jejunum and colon of the rabbit intestine. Mean ± S.E.(n=3).

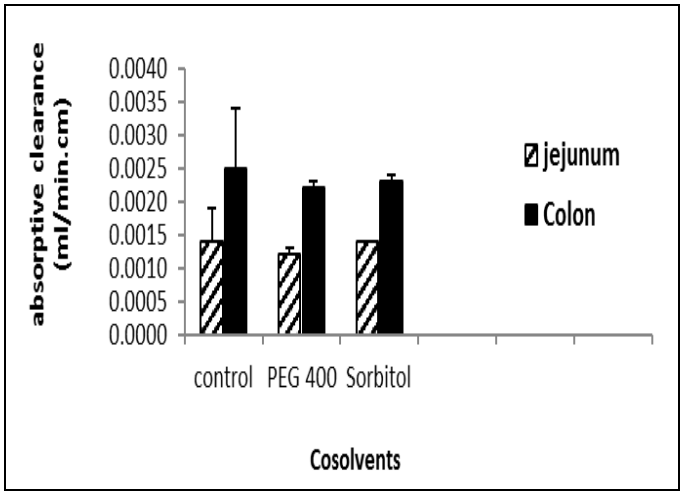


Figure 4. Effect of PEG-400 and Sorbitol on the absorptive clearance of ketotifen fumarate from jejunum and colon of the rabbit intestine. Mean ± S.E.(n=3).

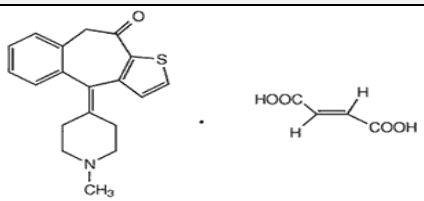
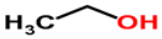
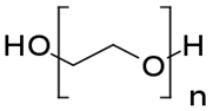
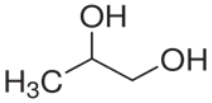
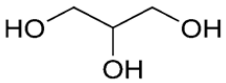
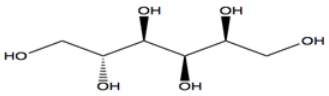
Name	Structure	Reference
Ketotifen fumerate		Adopted from Wyszomirska E., *et al.*, 2013) [32]
Ethyl Alcohol		Adopted from http: //www.drugbank.ca/drugs/DB00898) [33]
Polyethylene Glycol-400		Adopted from http: //www.drugbank.ca/drugs/DB11077)[34]
Propylene Glycol		Adopted from http: //www.drugbank.ca/drugs/DB01839)[35]
Glycerol		Adopted from http://www.drugbank.ca/drugs/DB09462) [36]
Sorbitol		Adopted from http://www.drugbank.ca/drugs/DB01638) [37]

Figure 5. Chemical structures of ketotifen fumarate and cosolvents investigated in the study[32-37]

Table 6. Effect of PEG-400 and sorbitol on membrane transport parameters of ketotifen fumarate in jejunum of rabbit intestine

*Parameter	Control	P.E.G-400 (40%)	Sorbitol 30%
Segment length (cm)	30	30	30
PeA	0.1421 **(0.0051)	0.0599 (0.0028)	0.0341 (0.0028)
Rout/Rin	0.5696 (0.0141)	0.8110 (0.0194)	0.9893 (0.0978)
PeA/L(ml/min.cm)	0.0051 (0.0001)	0.0022 (0.0001)	0.0014 (0.0001)
% Fa/cm	1.5584 (0.0518)	0.6808 (0.0530)	0.0914 (0.3675)
l*(L95%)(cm)	154.5784 (0.7677)	475.4730 (23.1623)	628.3498 (15.1697)
ARL (cm)	-34.5784 (22.71)	-355.473 (3.65)	-508.4398 (4.05)
JW(ml/min)	0.0375 (0.0146)	-0.1014 (0.0143)	-0.0169 (0.0003)
JW/L (ml/min.cm)	0.0014 (0.0005)	-0.0037 (0.0005)	-0.0006

Parameters are the same of **Table-1*
***Values between brackets are ± standard error, n = 3.*

Table 7. Effect of PEG-400 and sorbitol on membrane transport parameters of ketotifen fumerate in ascending-colon of rabbit intestine.

*Parameter	Control	P.E.G-400 (40%)	Sorbitol 30%
Segment length (cm)	**15**	**15**	**15**
PeA	0.0954 **(0.0107)	0.0401 (0.0005)	0.0311 (0.0008)
Rout/Rin	0.6874 (0.0369)	0.8540 (0.0242)	0.9067 (0.0044)
PeA/L (ml/min.cm)	0.0071 (0.0003)	0.0027 (0.0001)	0.0023 (0.0001)
Fa/cm	2.3309 (0.1276)	0.9930 (0.1526)	0.6827 (0.0027)
l* (L95%) (cm)	118.0405 (9.3394)	422.6407 (12.2996)	464.2081 (11.3692)
ARL (cm)	-103.0405	-407.6407	-449.2081
JW (ml/min)	0.0342 (0.0144)	-0.0735 (0.0047)	-0.0951 (0.0144)
JW/L (ml/min.cm)	0.0025 (0.0009)	-0.0050 (0.0002)	-0.0071 (0.0013)

Parameters are the same of **Table 1*
***Values between brackets are ± standard error, n = 3.*

PeA/L by 68.63 % and 76 %; while PG had increased the PeA/L by 47.06 % and 29.58 % in both segments respectively.

The major reason for this increase of the absorptive clearance upon addition of ethanol, glycerol, and propylene may be due to membrane fluidization effect of these cosolvents and disruption of the lipid bilayer structure which enhances transcellular absorption of ketotifen fumarate[26-29].Sinceour results had shown that ketotifen fumarate is dependent mainly on the transcellular diffusive pathway in its transport across the intestinal membrane (**Table 3**) so that these cosolvents had a highly significant effect on the transcellular absorption of this compound in both

Table 8. Estimated number of H-bounds

Cosolvent	*Estimated numberof H-bounds
P.E.G 400	8
P.G	4
Ethanol	2
Glycerol	6
Sorbitol	12

**H-bond Capacity according to Stein's Rule*[31]

jejunum and ascending colon. The second reason is that ketotifen fumarate is a lipophilic drug with log p = 2.2[6]. So that it easily partitioned into the phospholipid structure of the cell membrane resulting in an increase in the transcellular diffusive absorption. Furthermore, both ethanol and glycerol are potent inhibitors to p-glycoprotein [25-28]. This inhibits the back efflux of the drug to the intestinal lumen with subsequent increase in absorptive clearance of ketotifen fumarate.

Inhibitory effect of PEG-400 and Sorbitol:

The effect of both PEG-400, Sorbitol on membrane transport parameters of ketotifen fumarate from jejunum and ascending colon of the rabbit intestine are depicted in **Figure 4**, and **Tables 6** and **7**. Both cosolvents decreased absorptive clearance(PeA/L) of ketotifen fumarate from both segments. The decrease in PeA/L in presence of both PEG-400 and sorbitol was statically different from the control (P-value < 0.05), and the percentage fraction absorbed per unit length (%Fa/cm) of ketotifen fumarate was also decreased (p-value < 0.05). The inhibitory action of cosolvents on membrane transport of ketotifen fumarate was in the order: sorbitol (40 %)>PEG-400 (40 %).

The percent decrease in the absorptive clearance of ketotifen fumarate in jejunum and ascending colon after addition of 40% sorbitol was 72.55% and 67.6% respectively and after addition of 40%, PEG-400 was 56.86% and 61.97% respectively.
Sorbitol and PEG-400 resulted in a decrease of intestinal absorption and permeability of ketotifen fumarate due to osmotic load which increased water secretion(JW/L) in the intestinal lumen, this was evident from the negative sign of Jw/L (**Tables 6** and **7**). The increase in the volume of gastric fluid was reported to enhance gastric emptying rate and reduce transit time in small intestine with subsequent decreases in the bioavailability of class III BCS drugs[30].Another reason is that according to stein rule[31], the estimated number of hydrogen bonds for ketotifen fumarate with sorbitol was 12 while that with PEG-400 was 8 reflecting that ketotifen fumarate forms H-bound complex to higher extent with sorbitol (**Table 8**) and (**Figure 5**). So that the inhibitory action of sorbitol on the transport of ketotifen fumarate was higher compared withPEG400. Another reason for the inhibitory effect of PEG400is that: PEG400 was found to decrease the thermodynamic activity of drug molecule like carbamazepine in the rabbit [38] by the same way it could reduce thermodynamic activity of ketotifen fumarate in the intestinal lumen with subsequent reduction of its absorptive clearance.

CONCLUSION

The results reveal that: the absorption of ketotifen fumarate was in the order ascending colon> duodenum > jejunum> ileum. This compound is dependent mainly on transcellular diffusive pathway during its transport across the intestinal membrane in all anatomical sites studied. Ethanol, glycerol, and propylene glycol had an enhancing effect on the absorption of ketotifen fumarate in both jejunum and ascending colon, this effect was in the order; ethanol 15 % >glycerol 30 % > propylene glycol (PG 40 %), While polyethylene glycol 400 (PEG-400 40 %) and Sorbitol 40 % had an inhibitory effect on the absorption of ketotifen fumarate in both jejunum and ascending colon, this effect was in the order: sorbitol (40 %) > PEG-400 (40 %).

Based on our results we suggest that: The use of ethanol, propylene glycol and glycerol as cosolvents in development and formulation of liquid oral dosage forms of ketotifen fumarate would enhance both the aqueous solubility and intestinal absorption of this drug, however both polyethylene glycol-400 (PEG-400) and sorbitol had an inhibitory effect on the intestinal absorption of ketotifen fumarate, so that they are not suitable to be used with this drug.

Addition of p-gp inhibitors in oral dosage forms would enhance the intestinal absorption of this drug since it showed the least absorption from the ileum, a segment rich in p-gp transporters. Finally, since ketotifen fumarate showed highest absorption from ascending colon, it would be of significant importance to develop colon-specific drug delivery systems or mucoadhesive systems of this drug to improve its intestinal absorption.

Authors contribution

All authors have equal contribution to the work in this research.

Conflict of Interest

The authors declare that they do not have any conflict of interest.

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