

Research Article



EFFECT OF SOME POLYMERS USED IN PREPARATION OF TABLETS ON ABSORPTION OF ACTIVE INGREDIENTS

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ABSTRACT

In this study, the effect of long term use of tablets containing hydrophilic polymer hydroxypropylmethylcellulose (HPMC) and plastic polymers (ethylcellulose and Eudragit RL PO) on absorption of Diltiazem hydrochloride was evaluated by using non-everted intestinal gut sac model and by giving rabbits these polymers for a month to evaluate the intestinal histological changes that may be caused by these polymers. For this purpose, Diltiazem HCl was used with various amounts of these polymers. Permeability of Diltiazem HCl was measured by taking samples at specific intervals for 4 hours, and determining the concentrations by UV-visible spectrophotometer at wave length of 237nm. *In vivo* study was applied by dividing rabbits into four groups (six animals in each group). Rabbits in the first group were served as normal and received water and food only. Rabbits in the second group received hydroxypropylmethylcellulose (20 mg/kg of body weight) for a month. Rabbits in the third group received eudragit RL PO (20 mg/kg of body weight) for a month. Rabbits in the fourth group received ethylcellulose (15 mg/kg of body weight) for a month also. Then the intestine was examined histologically. Results showed that the cumulative concentrations of these polymers decreased the absorption of Diltiazem HCl through the intestine by this sequence: ethylcellulose > Eudragit RL PO > HPMC due to histological alterations that were evaluated after *in vivo* study which indicated that ethylcellulose caused marked morphological changes on tissues. Eudragit RL PO caused morphological changes ranged from moderate to marked changes, and hydroxypropylmethylcellulose caused mild to marked morphological changes on intestinal tissues.

Keywords: Diltiazem hydrochloride, hydroxypropylmethylcellulose, Eudragit RL PO, Ethylcellulose, non-everted gut sac model, permeability, histological alterations.

INTRODUCTION

Diltiazem hydrochloride is a calcium channel blocker used in the treatment of angina, heart arrhythmia and high blood pressure. It has a short half life (3-5 hours), so its frequency of administration is not less than 3-4 times daily. This indicates that giving it as modified release dosage forms is more appropriate.^{1,2}

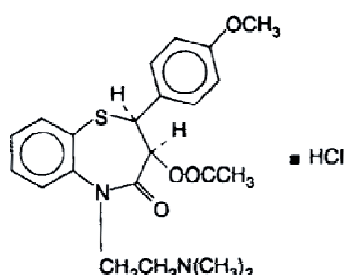


Figure 1: Structure of Diltiazem HCl.

Usually for preparing these dosage forms some polymers are used as follows:

1. Hydrophilic and pH independent polymer; hydroxypropylmethylcellulose is used at concentrations of 10-80% w/w to retard the drug release from these dosage forms.
2. Plastic polymers; ethyl cellulose (EC) is used at concentrations of 15- 35% w/w, and Eudragit RL PO is used at concentrations of 35- 45% w/w.

The aim of this study was to evaluate the effect of long term use of these polymers on the absorption of Diltiazem HCl from intestinal wall into blood circulation, and to determine whether there is a need to increase the dose of Diltiazem HCl after a specific period of time.

MATERIALS AND METHODS

Animals: White male New Zealand rabbits weighing 2.5-3 kg obtained from Leen institute for experimental animals and animal Facility, Faculty of Pharmacy, Damascus, Syria, were selected for the present study. Animals were housed in an air conditioned animal house facility at 25±1°C, with 50%±5% relative humidity under a controlled 12 h light/dark cycle. Food and tap water were provided.

Test chemicals: Diltiazem hydrochloride was purchased from Menarini group pharmaceutical company, Italy.

Hydroxypropylmethylcellulose (HPMC K4M) were purchased from REDA industrial materials, United Arab Emirates, Ethyl cellulose from HANGZHOU ZHONGBAO IMP.EXP. CO. LTD, China.

And Eudragit RL PO was purchased from Evonik industries, Germany.

In Vitro Experimental Design: We studied the permeability of Diltiazem HCl by using non-everted rabbit's intestinal gut sac model³⁻⁵. The rabbits were fasted all over the night without preventing them from



drinking water. Next day, they were sacrificed and the distal part of jejunum was isolated within ten minutes because the bioavailability of Diltiazem HCl from this part is better than from the first 30 cm (the proximal part) of the small intestine of rabbits⁶, and the mechanism of its absorption is passive transepithelial across intestinal tissue leading to tight junctions opening⁷, Diltiazem HCl was dissolved in phosphate buffered normal saline (PBS pH= 7.4). The solution was injected inside the lumen of the distal part of jejunum. Permeability of Diltiazem HCl was measured versus time by taking samples from medium of serosal side (PBS pH= 7.4) and determining their concentrations using UV-visible spectrophotometer (HITACHI 1800U) at the wave length 237nm.

Phosphate buffered normal saline (pH= 7.4) was used for permeability studies inside and outside the lumen, and for washing the isolated part to remove blood.

Surface area of the segments was constant for all experiments (25 cm²). Formulas described in table 1 were dissolved in 7 ml of PBS (pH= 7.4), and injected inside the lumen of the intestine.

Table 1: Formulas used in the permeability studies

Group N	Diltiazem HCl (mg)	-
N	24	-
Group A	Diltiazem HCl (mg)	HPMC (mg)
A1	24	25
A2	24	35
A3	24	45
Group B	Diltiazem HCl (mg)	Eudragit RL PO (mg)
B1	24	35
B2	24	40
B3	24	45
Group C	Diltiazem HCl (mg)	Ethylcellulose (mg)
C1	24	15
C2	24	25
C3	24	35
Group D	Diltiazem HCl (mg)	HPMC (mg)
D1	36	45
D2	48	45
Group E	Diltiazem HCl (mg)	Eudragit RL PO (mg)
E1	36	45
E2	48	45
Group F	Diltiazem HCl (mg)	Ethylcellulose (mg)
F1	36	35
F2	48	35

Both ends of the segment were tighten, and it was placed in 200 ml of PBS (pH= 7.4) with stable temperature of 37°C and stable carbogen (95% O₂+ 5% CO₂) flow rate, and incubated for 4 hours in an oscillating bath (75 cycle per minute) using magnetic stirrer (BOECO MSH 300, Germany). The percentage of drug permeability was determined versus time. At the end of incubation time, sacs were cut, fixed in formalin, embedded in paraffin,

and studied under microscope to determine the histological changes.

In Vivo Experimental Design: Rabbits were divided into four groups (six animals in each group).

Rabbits in the first group were served as normal and received water and food only. Rabbits in the second group received hydroxypropylmethylcellulose (20mg/kg/day) for a month. Rabbits in the third group received eudragit RL PO (20 mg/kg/day) for a month. Rabbits in the fourth group received ethylcellulose (15 mg/kg/day) for a month. At the end of the experimental period, intestines were isolated and studied histologically.

Determination of Diltiazem Hydrochloride Concentrations:

The concentrations of Diltiazem HCl were measured by UV-visible spectrophotometer at the wave length 237 nm.

Histological study: Immediately after sacrifice, the specimens were fixed in 10% formalin and submitted to histological study. After embedding the tissue in paraffin blocks, Sections at 5 microns were cut with a microtome (Leica RM 2155, Germany), sections were prepared and stained with hematoxylin - eosin and interpreted using light microscope (Nikon 273324, Japan). The entire histological evaluation was performed by a histopathologist who was blinded to group distribution when this analysis was applied.

Statistical Analysis: Data are presented as means± SD.

Significance of differences between groups was estimated by student's t-test.

P< 0.05 was considered statistically significant.

RESULTS

Analysis of absorption

These polymers (HPMC, Eudragit RL PO, and EC) were found to significantly (P< 0.05) reduce the permeability of Diltiazem HCl as the amounts of polymers increase. EC was the most polymers that reduce the permeability rate of Diltiazem HCl, unlike HPMC which was the least influential on the permeability rate as described in table 2. The results in table 2 are described in figures 2, 3, 4, 5, 6, 7.

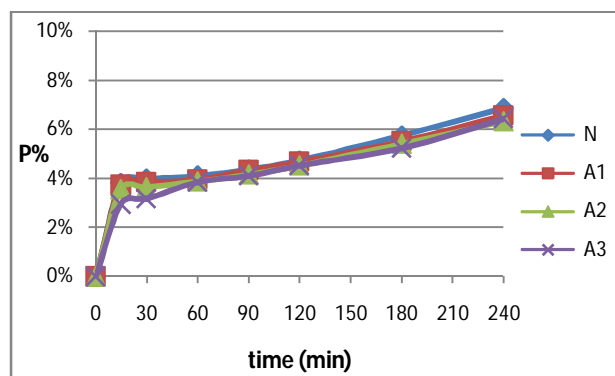


Figure 2: Permeation rate of Diltiazem HCl from A formulas compared with N formula.



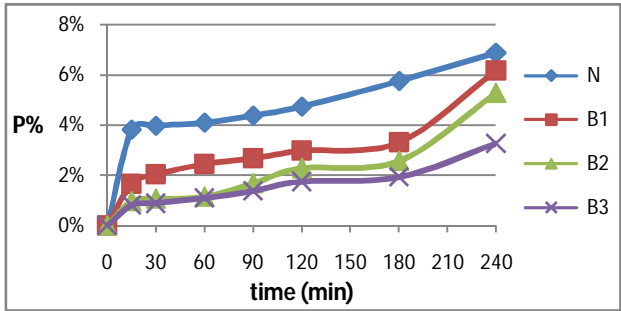


Figure 3: Permeation rate of Diltiazem HCl from B formulas compared with N formula.

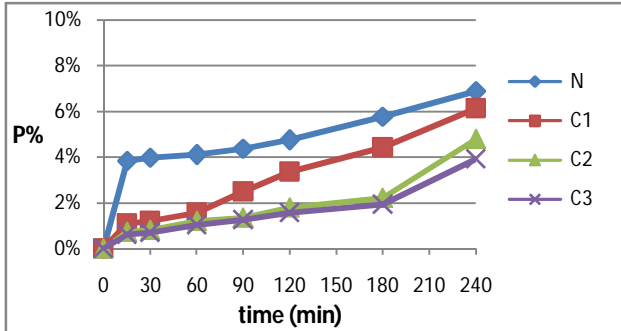


Figure 4: Permeation rate of Diltiazem HCl from C formulas compared with N formula.

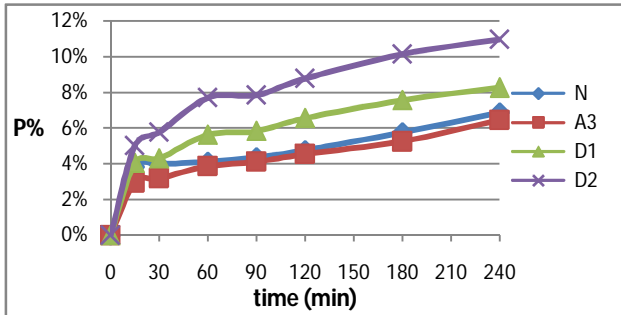


Figure 5: Permeation rate of Diltiazem HCl from D formulas compared with N formula.

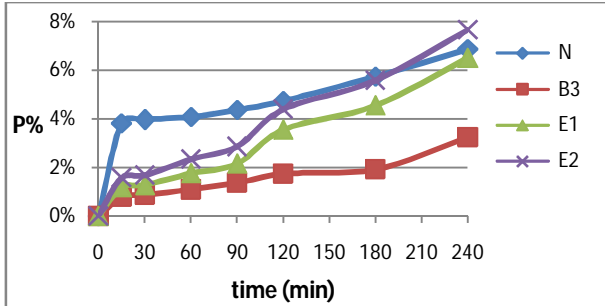


Figure 6: Permeation rate of Diltiazem HCl from E formulas compared with N formula

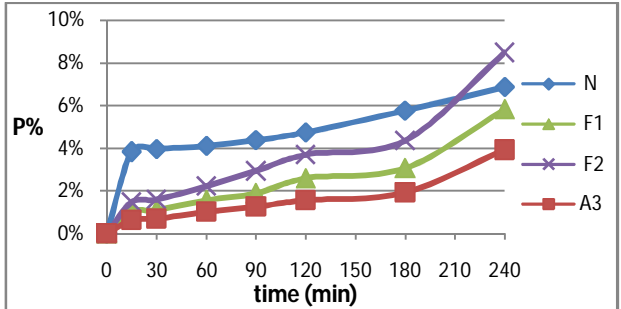


Figure 7: Permeation rate of Diltiazem HCl from F formulas compared with N formula

Table 2: Results of permeability studies

Group N	Average of permeability ratio (P %)
N	4.20
Group A	P%
A1	4.08
A2	3.94
A3	3.77
Group B	P%
B1	2.66
B2	1.87
B3	1.39
Group C	P%
C1	2.53
C2	1.60
C3	1.38
Group D	P%
D1	5.27
D2	7.02
Group E	P%
E1	2.63
E2	3.27
Group F	P%
F1	2.13
F2	3.09

The histological analysis

The histological changes were evaluated after the *in vitro* and *in vivo* studies, but the evaluation was more credible after *in vivo* studies so it has been adopted.

A set of criteria was relied for signs of mal-absorption to assess the morphological changes occurring to intestinal tissue: villi, crypts, inflammatory infiltrate, mucus secretion and necrosis of the tissues, these are classified according to their severity as follows: not evident, mild, moderate, marked and severe as shown in table 3.

Histological changes caused by these polymers after *in vivo* study were described in figures (8, 9, 10, 11) that show a decrease in the villi's lengths and counts associated with cryptic proliferation (hyperplasia), abnormalities in mucus secretion and intraepithelial lymphocytes counts compared with control group, all these are morphological features that could indicate formal-absorption compared with the normal sample which was taken from the same part of the first group of rabbits.

Table 3: Results of histological evaluation after *in vivo* study

Group	Not evident	mild	Moderate	marked	Severe
1	+				
2		+		+	
3			+	+	
4				+	



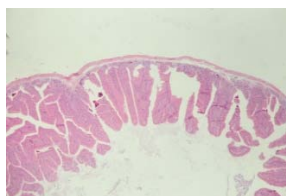


Figure 8: The small intestine histology of the control group (Group 1).

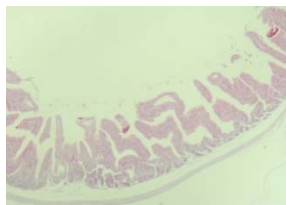


Figure 9: The histological changes of the small intestine of group 2.

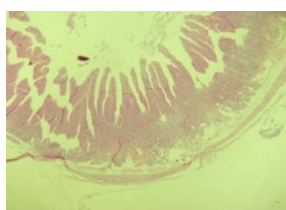


Figure 10: The histological changes of the small intestine of group 3.

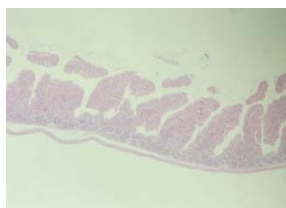


Figure 11: The histological changes of the small intestine of group 4.

DISCUSSION

The study was divided into two stages; the first stage was the determination of Diltiazem HCl permeation rate which is from the first class of drugs⁹ after application of polymers (HPMC, Eudragit RL PO and EC), that decreased the permeation rate of the drug significantly compared with the permeation rate of the drug without the application of polymers by the following sequence: EC> Eudragit RL PO> HPMC, this is because of histological changes and their adherence to the intestinal wall which could be interpreted by six theories¹⁰:

Electrical, diffusion, wetting, adsorption, mechanical and cohesive theories that caused by the formation of hydrogen, Vander Waals and hydrophobic bonds which are classified as physical bonds, and by forming covalent and ionic bonds that are classified as chemical bonds.

The residence time of the polymers played a main role on their adhesion to the intestine.

The second stage was the in vivo study on rabbits; the highest concentration of the polymer that caused a lack of permeation rate by the permeation studies previously was given for one month. The histological changes were

studied under light microscope by a pathologist blinded to the group distribution for three times.

The used polymers may adhere to the intestinal wall tightly by one of the six theories of adherence leading to a decrease in the lengths of villi and their counts associated with abnormalities of mucus secretion and intraepithelial lymphocytes counts, all these could be indices of malabsorption cases that lead to a decrease of active ingredient absorption after a long period of time.

HPMC is a non ionic hydrophilic polymer; it is easy to get rid of it by mucus intestinal renewing as it is soluble in water.

It causes mild to marked histological changes due to its adherence that could be less than hydrophobic polymers.

Eudragit RL PO is a cationic and hydrophobic polymer; it will be attracted to the negatively charged intestinal wall and remains held tightly until another secretion cleans it away leading to moderate to marked histological changes.

EC is a non ionic hydrophobic polymer; it caused marked histological alterations of the intestinal mucosa, it could have high ability to adhere on the intestinal wall that makes it hard to be cleaned easily by mucus renewing and caused this changes.

CONCLUSION

In this study, the used polymers have decreased the absorption of Diltiazem HCl from intestinal wall as follows: HPMC< Eudragit RL PO< EC due to their adhesion and to the histological alterations of the intestinal mucosa that developed after long-term use and affected the active ingredients absorption. These results indicate the necessity to increase the dose of active ingredients after specific period of time to obtain the desired pharmacological effect.

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