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Formulation and *In-Vitro*, *Ex-Vivo*, and *In-Vivo* Evaluation of Mucoadhesive Buccal Tablets Containing Labetalol Hydrochloride for Enhancement of Systemic Bioavailability

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ABSTRACT

Objective: Labetalol hydrochloride is an alpha/beta adrenoceptor blocker that undergoes comprehensive first pass-metabolism resulting in a low oral bioavailability. This study aimed to formulate and evaluate mucoadhesive buccal formulations of labetalol hydrochloride for enhancement of its bioavailability. **Methods:** Using various concentrations of hydroxypropyl methylcellulose (HPMC), carbopol-934, and sodium alginate, ten formulations of mucoadhesive buccal tablets containing labetalol hydrochloride were prepared. The produced tablets were evaluated to test physical and mucoadhesive properties as well as in-vitro drug release properties. Ex-vivo evaluations of the tablets were examined using chicken pouch membrane. Formulations that offered best results in in-vitro and ex-vivo evaluations were selected for running in-vivo comparative bioavailability study using New Zealand rabbits and adopted HPLC method to assess the buccal bioavailability of labetalol hydrochloride in relation to its oral bioavailability from commercial tablets. **Results:** It was found that drug release and mucoadhesive properties depended on the type and proportion of different polymers. Sodium alginate-containing formulations showed higher release rates and ex-vivo permeation rates compared to carbopol-containing formulations. Increasing the proportion of HPMC resulted in more swelling, better mucohesion forces and times but more delayed permeation and release rates. A strong correlation was detected between in-vivo drug release and ex-vivo transmucosal permeation of labetalol hydrochloride. The relative bioavailability of labetalol hydrochloride from the selected mucoadhesive buccal tablets F1 and F6 were 2.76 and 1.60, respectively. **Conclusion:** The produced mucoadhesive buccal tablets were successful in improving the systemic bioavailability of labetalol hydrochloride in rabbits. Clinical applications of formulations F1 and F6 are recommended.

Keywords: Mucoadhesion, rabbits; Relative bioavailability; Carbopol-934; HPMC; Sodium alginate.

INTRODUCTION

Delivery of drugs in the oral cavity can be classified according to site of application into local, buccal and sublingual delivery. Buccal delivery involves

the absorption of drugs through buccal mucosa that can offer both local and systemic effects due to highly vascularized tissues¹. The buccal route is preferred over the oral route for drugs that suffer from acid decomposition in the stomach or that are highly

metabolized in the liver (through first pass effect); as the drug is absorbed directly into systemic circulation from buccal mucosa through jugular vein ^{2,3}. Buccal route of drug delivery also has the advantages of suitability for unconscious patients. Besides, therapy can be terminated at any time by detaching the dosage form out of the oral cavity if required so drug toxicity can be controlled ^{4,5}. Patients are generally more comfortable and compliant using buccal route of drug delivery than other penetrative routes ⁶.

Mucoadhesion properties are introduced by using mucoadhesive polymers in buccal preparation systems. These polymers have numerous hydrophilic groups (hydroxyl, amide, carboxyl and sulphate) upon hydration they cause polymer to swell and become adherent to the buccal mucosa by various interaction forces like (hydrogen bonding and hydrophobic or electrostatic interactions) ⁷. Mucoadhesive polymers include natural polymers as sodium alginate, synthetic polymers as carbopol-934, and semisynthetic polymers as hydroxypropyl methylcellulose (HPMC) ³. Different dosage forms are suggested for buccal mucosal delivery including tablets, films/patches, lozenges, and gels ^{3,8}. Buccal tablets are thin and small in size, unlike traditional oral tablets, they allow drinking and speaking. They softened and bound to the mucosal membrane and remain in place for sufficient time to allow full drug release ^{9,10}.

Labetalol hydrochloride is an antihypertensive agent that inhibits the activity of both alpha and beta adrenergic receptors. It is prescribed for the treatment of acute or chronic vascular hypertension ¹¹. After oral administration, the drug is nearly completely absorbed from the gastrointestinal (GI) tract, but in the liver and/or GI mucosa, the drug undergoes significant first-pass metabolism. It is characterized by a short biological half-life of 2-5 hours and it undergoes significant pre-systemic metabolism resulting in low bioavailability 25% ¹². It has a low molecular weight (364.9 g/mol) and a favorable partition coefficient (7.08) ¹¹ so it is small and lipophilic enough to pass through buccal mucosa. The current study hypothesized that buccal administration of labetalol hydrochloride may enhance the systemic bioavailability of the drug as it avoids the pre-systemic metabolism.

This study aimed to formulate and characterize mucoadhesive buccal tablets containing labetalol hydrochloride using different combinations of mucoadhesive polymers in order to enhance the systemic bioavailability. The characterization processes included physical, in-vitro, ex-vivo, and in-vivo evaluation.

MATERIAL AND METHODS

Labetalol hydrochloride was kindly received from Al Debeiky Pharma (DBK) Pharmaceutical Co.

(El Nozha El Gdida, Cairo, Egypt). Carbopol-934, hydroxypropyl methylcellulose (HPMC), and sodium alginate were supplied from Global Napi pharmaceutical Co. (ElKattamya, Cairo, Egypt). Potassium dihydrogen phosphate and disodium hydrogen phosphate were obtained from El-Nasr Pharmaceutical Chemicals Co. (Cairo, Egypt). Ammonium phosphate and ammonium acetate ortho phosphoric acid were obtained from Lanxess Energizing Chemistry Co., Germany. HPLC grade of diethyl ether and methanol were purchased from Merck, Germany. Labipress 100 tablets (DBK Pharmaceutical Co., El Nozha El Gdida, Cairo, Egypt) were used as reference oral tablets in the current study. All other chemicals were of analytical grade.

Methodology

Standard plot of labetalol hydrochloride in phosphate buffer pH 6.8

Labetalol hydrochloride was dissolved in 50 ml phosphate buffer pH 6.8 to produce stock solution having a concentration of 400 µg/ml. From the prepared stock solution, suitable serial dilutions were prepared using phosphate buffer pH 6.8 in the range of 10-100 µg/ml. Using a UV-visible spectrophotometer one of the produced dilutions was scanned between 400 and 200nm to determine the λ max of the drug. At this determined wavelength, the absorbance of all the other dilutions was measured against phosphate buffer pH 6.8 (blank solution). The absorbance versus concentration (µg/ml) was plotted and subjected to linear regression analysis where values of intercept and slope were noted.

Calculation of the dose of labetalol hydrochloride in the buccal tablets

Taking into account that the first-pass effect will be skipped in the buccal route, the dose of Labetalol hydrochloride incorporated in buccal tablets should be lower than that used in oral tablets. The buccal route is proposed to achieve 100% bioavailability while the oral bioavailability of Labetalol hydrochloride from conventional tablets is 25% ¹³. Conventional tablets of Labetalol hydrochloride are available in 100 or 200 mg dose. So it was decided to include 25 or 50 mg of drug in the mucoadhesive buccal tablets.

Drug excipient compatibility studies

Fourier Transform Infrared Spectroscopy (FTIR) was used to detect any physicochemical interactions between Labetalol hydrochloride and the used excipients. Labetalol hydrochloride alone and in a physical mixtures with HPMC and either sodium alginate or carbopol-934 were prepared and then mixed with a suitable amount of potassium bromide. The mixture was compressed into pellet and scanned using a FTIR spectrophotometer (Nicolet iS10, Thermo Fisher Scientific, U.S.A) over a wave number ranging from

4000 to 400 cm^{-1} . The produced IR spectrums of the mixtures were compared with that of Labetalol hydrochloride alone and the peaks were matched to detect any differences. The IR spectrums of each of the polymers alone were also produced to allow for comparisons.

Differential Scanning Calorimetry (DSC) investigation was done using Mod.: DSC-60A, Shimadzu, Japan. Samples weighting 1.5 to 4 mg were used in Aluminum Cell. Operating conditions included ambient temperature up to 500 °C using liquid nitrogen at a flow rate of 30 ml/minute. Heat was increased at a rate of 10 °C/minute.

Preparation of labetalol hydrochloride mucoadhesive buccal tablets

Mucoadhesive buccal tablets containing 25 mg labetalol hydrochloride were prepared by direct compression method. Bioadhesive polymers, namely, HPMC, carbopol-934 and sodium alginate were used to offer the mucoadhesive properties to the tablets. Ten different formulations were prepared as shown in **Table 1**. Different proportions of HPMC were used with 25 mg Carbopol-934 or sodium alginate. Magnesium stearate was used for lubrication (4 mg) and talc was added as a glidant (1 mg). Lactose was used as a diluent to complete the weight to 150 mg. The powder portions were sieved and mixed geometrically using a pestle and a mortar. The produced powder blend was directly compressed using 7mm a single flat faced punch tablet machine (AR 400, Erweka Apparatebau, Germany). The produced tablets were stored for further evaluation.

***In-vitro* evaluation of mucoadhesive buccal tablets**

Hardness, weight variation, friability, and surface pH

The hardness of five buccal tablets from each patch was measured using a hardness tester (TB 24, Erweka Apparatebau, Germany). Hardness was presented in kg/cm^2 .

Using electronic digital balance (EB, Poland), ten buccal tablets from each patch were weighed individually and the mean weight was calculated.

A friabilator (TA3R, Erweka Apparatebau, Germany) was used to measure the friability of the produced tablets. Pre-weighed ten buccal tablets from each patch were placed in the friabilator which revolves 25 revolutions per minute. After 4 minutes in the apparatus, the tablets were de-dusted and reweighed. The percentage weight loss was determined.

Five buccal tablets from each batch were randomly selected. The tablets were finely crushed in a mortar. An amount of 150 mg was mixed with 50 ml of phosphate buffer pH 6.8; stirred on magnetic stirrer for 1 hr. and then transferred to 100 ml volumetric flask completed with phosphate buffer to the mark. Samples were withdrawn, filtered through Millipore filter (0.45

μm). After suitable dilution, the sample was analyzed spectrophotometrically at 304 nm. Each measurement was repeated three times; the mean results and standard deviation (SD) were obtained.

One ml of distilled water was added to the tablets for two hours to allow tablets' swelling. The pH of the surface of the swollen tablets was measured by placing the electrode on the surface and allowing it to achieve equilibrium for one minute.

Swelling index

Three mucoadhesive buccal tablets from each patch were weighed (W_1) then placed in a petri dish containing 10 ml phosphate buffer pH 6.8. The tablets were removed every hour and excess water on the surface were gently dried, reweighed (W_2) and then returned back to the dish. The process continued for 8 hrs. The swelling index (SI) was calculated by the formula; $SI = \frac{W_2 - W_1}{W_1} \times 100$.

***In-vitro* drug release study**

USP cell dissolution type II apparatus (Abbott 8, USA) was used to study the release of labetalol hydrochloride from the produced buccal tablets. The dissolution medium (900 ml of phosphate buffer pH 6.8) was kept at $37 \pm 0.5^\circ\text{C}$ with a rotating speed of 50 rpm. The tablet was glued (from one side) to a glass slide which was then placed in the dissolution vessel with the tablet facing the upper side of the vessel. The experiment was run for eight consecutive hours. Samples (5 ml) were withdrawn each hour and replaced with equal volume of fresh phosphate buffer pH 6.8 warmed at 37°C . The samples were filtered using Millipore filter then analyzed spectrophotometrically at 304 nm.

To explore the kinetic behavior of labetalol hydrochloride release from the mucoadhesive buccal tablets, different models were applied including zero order, first order, Higuchi, and Korsmeyer peapas models. The model which reveals the best fit to the release data represents the kinetic behavior of the drug.

***Ex-vivo* evaluation of mucoadhesive buccal tablets**

Ex-vivo evaluation was conducted using chicken pouch membrane^{14,15}. Fresh clean membranes were brought to the laboratory, directly after slaughtering, in a saline solution. After removal of all adjacent tissues and fats the chicken pouch membrane was carefully washed with normal saline solution and was either directly used for running the experiments or stored at -20°C and allowed to defreeze just before the experiment.

***Ex-vivo* mucoadhesion time**

A piece of chicken pouch membrane of about 4-5 cm long was glued to a glass slide with the mucosal side facing the top. One surface of the tested tablet was

Table 1. Compositions of the produced formulations of mucoadhesive buccal tablets

Formulation code	Ingredients (mg)						
	Drug (LH)	HPMC	Sodium alginate	Carbopol-934	Magnesium stearate	Talc	Lactose
F1	25	0	0	25	4	1	95
F2	25	12.5	0	25	4	1	82.5
F3	25	25	0	25	4	1	70
F4	25	37.5	0	25	4	1	57.5
F5	25	62.5	0	25	4	1	32.5
F6	25	0	25	0	4	1	95
F7	25	12.5	25	0	4	1	82.5
F8	25	25	25	0	4	1	70
F9	25	37.5	25	0	4	1	57.5
F10	25	62.5	25	0	4	1	32.5

LH: labetalol hydrochloride, and HPMC: hydroxypropyl methylcellulose.

hydrated with phosphate buffer pH 6.8 then fingertips pressure was applied for 30-40 seconds to stick that surface of the tablet to the mucosal membrane. USP disintegration apparatus (ZT3, Erweka Apparatebau, Germany) was used with modification to suit this experiment. The medium used consisted of 800 ml phosphate buffer pH 6.8 kept at $37 \pm 0.5^\circ\text{C}$ and slowly stirred to resemble the buccal conditions. The glass slide carrying the tablet was then fixed to the disc of the apparatus which was moving up and down where the tablet was completely immersed in the medium. Ex-vivo mucoadhesion time was measured as the time taken by the tablet to completely detach from the mucosal membrane. The experiment was performed three times with fresh chicken pouch membrane each time and the mean mucoadhesion time and SD were calculated.

Ex-vivo mucoadhesive strength

The modified physical balance method was used to measure the ex-vivo mucoadhesive strength of labetalol hydrochloride mucoadhesive buccal tablets^{16,17}. A piece of fresh chicken pouch membrane was stretched over the orifice of a glass vial and fixed in place by a rubber band. The vial is placed in a glass beaker filled with phosphate buffer solution pH 6.8 to the level of the membrane. The two pans of the balance were replaced with 2 beakers; the left pan was replaced with a beaker containing water while the right pan was replaced with a beaker which had the tablet glued to its bottom. Then both arms were balanced by adding an appropriate weight. The beaker containing the vial with the membrane was then placed under the tablet so that the tablet was sandwiched between the upper beaker and the membrane. A 50 gram load was placed in the upper beaker for one minute to allow the tablet to stick to the membrane after which this load was removed. Drops of

water were added at a constant rate in the left side beaker gradually until the tablet is detached from the membrane. The weight of the added water necessary to detach the tablet from the membrane represented the mucoadhesive strength. The process was repeated three times with fresh membrane each time and the average weight was reported. The force of adhesion was calculated using the following formula; force of adhesion (dyne) = 981 x (mucoadhesive strength (gram)).

Ex-vivo transmucosal permeation study

The ex-vivo permeation of labetalol hydrochloride from bucoadhesive tablets through chicken pouch membrane was studied using the procedure previously described by Tayel, et al., 2010¹⁸. The procedure involves a modification of the of the USP dissolution apparatus method where a donor chamber is created to resemble the conditions in buccal cavity. The donor chamber consisted of a small glass tube opened from both upper and lower ends. One end of the glass tube was covered with a piece of chicken pouch membrane that was fixed tightly by a rubber band. To the chicken pouch mucosal side, bucoadhesive tablet was pressed for one minute to allow bonding. The donor chamber was then loaded with 2 ml phosphate buffer pH 6.8 to resemble the buccal cavity. The loaded donor chamber was attached to the shaft of USP cell dissolution apparatus (Abbota 8, USA) and immersed in the dissolution medium so that the membrane was just below the surface. The dissolution medium consisted of 100 ml phosphate buffer pH 6.8 maintained at $37 \pm 0.5^\circ\text{C}$, and the apparatus was rotated at 50 rpm for 8 hours. Samples of 5 ml were withdrawn each hour and equal volumes of fresh buffer were added for compensation. The samples were analyzed spectrophotometrically at 304 nm.

In-vivo bioavailability Study

The parallel design was used to conduct a comparative bioavailability study to examine the bioavailability of labetalol hydrochloride from the produced mucoadhesive tablets in comparison to that from available commercial tablets. According to the results of in-vitro dissolution, ex-vivo study and mucoadhesive characteristics of the ten produced formulations, the formulations F1 and F6 were chosen for running the bioavailability study as they offered the most optimum results for each of carbopol-943-containing formulations and sodium alginate-containing formulations, respectively. Commercial Labipress 100 oral tablets of labetalol hydrochloride were used as a reference. New Zealand rabbits were used as an animal model to run the bioavailability study. Ethical approval was granted from the Faculty of Pharmacy-Mansoura University-Ethical Committee to run this study.

Administration and blood collection

Twelve healthy male New Zealand rabbits weighing 1.5 –2.0 kg (average body weight of 1.75 kg) were used in this experiment. The rabbits were fasted overnight and kept in separate cages prior to running the experiment. The rabbits were anesthetized by an I.M. injection of a 5:1 mixture of ketamine (9.3 mg/kg) and xylazine (1.9 mg/kg) followed by inhalation of isoflurane.

The animals were divided into three groups of four rabbits each. The animals of first group received one quarter of commercial Labipress 100 oral tablet as a reference formulation. The second group of animals received F1 mucoadhesive tablet and third group received F6 mucoadhesive tablet. Following the induction of anesthesia, the mucoadhesive tablets were lightly pressed against the buccal mucosa of the oral cavity of the rabbit, in the area of the upper canine, between the gingiva and cheek.

Two millimeter blood samples were then withdrawn from the rabbits' ear vein each hour for six consecutive hours. The samples were collected in tubes containing ethylenediaminetetraacetic acid (EDTA). Blood samples were centrifuged for 10 minutes at 3000 x g to separate the plasma. The clear supernatant of serum layer was then collected in pre-labeled tubes and stored at -20 °C for further analysis.

Samples analysis

The quantitative determination of labetalol hydrochloride was performed using Perkin Elmer Series 200 Pump High-Performance Liquid Chromatography (HPLC) system (U.S.A). The mobile phase consisted of 1:1 mixture of 0.01M ammonium phosphate and methanol. The pH of the mobile phase was adjusted to 3.0 using 15M phosphoric acid. A flow-rate of one ml/min was used. The mobile phase was filtered through

a 0.45 µm Millipore filter, degassed by ultra-sonication and maintained at room temperature. The procedure for extraction and column injection was performed as described in detail by Hidalgo IJ and Muir KT in 1984¹⁹.

Pharmacokinetic analysis

Assuming one compartment model of drug distribution, different pharmacokinetic parameters were calculated. Pharmacokinetic parameters included the maximum blood concentration achieved (C_{max}), the time taken to reach this concentration (T_{max}), the elimination half-life time ($t_{1/2}$) of the drug, and the elimination rate constant (K) defined as $0.963/t_{1/2}$. The area under the curve (AUC) was used to represent the extent of drug absorption while the C_{max} and T_{max} were used to represent the rate of drug absorption. The AUC was calculated by the trapezoidal rule. Both AUC (0, 6 hrs) and AUC (0, inf.) were calculated.

The relative bioavailability (F) was calculated by the following formula:

$$F = \frac{\text{AUC (0 to inf.) for mucoadhesive buccal tablet}}{\frac{\text{AUC (0 to inf.) for oral tablet}}{\text{Dose of oral tablet}}} \times \frac{\text{Dose of mucoadhesive buccal tablet}}{\text{Dose of oral tablet}}$$

ANOVA test was used to examine the statistical significance of any differences between the pharmacokinetic parameters of labetalol hydrochloride buccal mucoadhesive tablets (F1 and F6) and the reference commercial oral tablets of labetalol hydrochloride.

RESULTS

Calibration curve

Labetalol hydrochloride λ max was detected at 304 nm. The linear regression equation of absorbance versus concentration ($\mu\text{g/ml}$) revealed an intercept of 0.0029 and slope of 0.0084.

Evaluation of drug excipient compatibility (FTIR and DSC analyses)

The results of FTIR spectroscopy are presented in **Figure 1**. No chemical incompatibilities were detected between labetalol hydrochloride and any of the polymer combinations. The characteristic peaks of labetalol hydrochloride; OH-stretching, NH-stretching, aromatic -CH, aliphatic-CH, C=O stretching, and C=C stretching were observed at 3354, 3188, 2977, 2804, 1674, and 1640 respectively. All the observed peaks were within the standard peaks ranges.

Figure 2 shows the DSC results of labetalol hydrochloride alone and in physical mixtures with HPMC and carbopol /sodium alginate. Labetalol

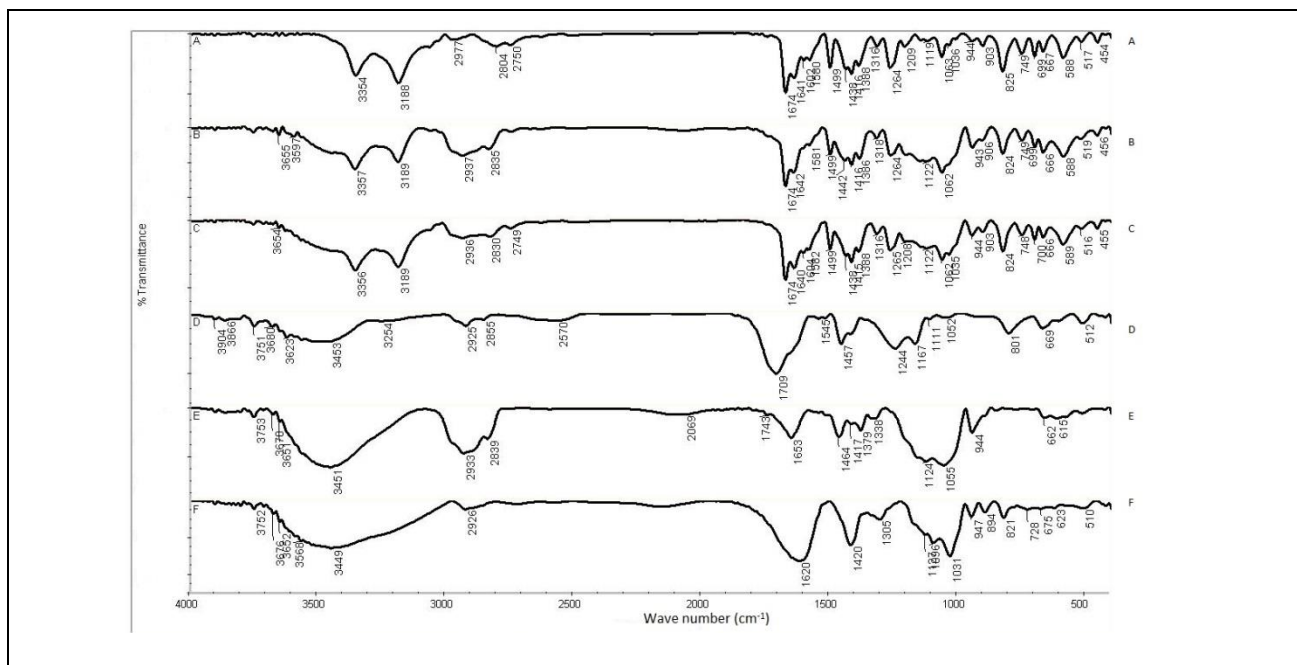


Figure 1. Fourier Transform Infrared Spectroscopy of (A) labetalol hydrochloride alone, (B) labetalol hydrochloride/ carbopol-934 / Hydroxypropyl methylcellulose (HPMC) mixture, (C) labetalol hydrochloride/ sodium alginate / HPMC mixture, (D) carbopol-934 alone, (E) HPMC alone, and (F) sodium alginate alone.

hydrochloride showed characteristic endothermic peak at 189.72°C representing the melting point. The melting point peak persisted when labetalol hydrochloride was mixed with HPMC and carbopol (186.75°C) and when mixed with HPMC and sodium alginate (188.78°C) reflecting no interactions. HPMC, carbopol and sodium alginate showed their characteristic peaks.

**In-vitro evaluation of mucoadhesive buccal tablets
Hardness, weight variation, friability, and surface pH**

The physical evaluations of the produced formulations are presented in **Table 2**. The mean ± SD hardness of carbopol-containing formulations (F1 to F5) was significantly higher (7.06 ± 0.11 kg/cm²) than that of sodium alginate-containing formulations (F6 to F10; 4.58 ± 0.15 kg/cm²; $P < 0.0001$). On the other hand, the mean ± SD percentage friability was lower in carbopol-containing formulations (0.14 ± 0.01) compared to sodium alginate-containing formulations (0.49 ± 0.03 ; $P < 0.0001$).

Carbopol-containing formulations showed lower mean ± SD surface pH (5.69 ± 0.78) compared to that of sodium alginate-containing formulations (7.69 ± 0.42 ; $P = 0.001$). The mean ± SD weight of the tablets ranged from 146.9 ± 3.3 to 156.6 ± 3.6 mg while the percentage drug content ranged from 94.4 ± 1.9 to 96.6 ± 5.5 %.

Swelling index

Figure 3 shows the swelling profiles for different produced formulations (F1 to F10). It was noted that formulations containing higher proportions of HPMC showed higher swelling indices values. Carbopol-containing formulations showed slightly higher swelling indices compared to sodium alginate-containing formulations when the ratio of HPMC is constant. Formulation containing 62.5 mg HPMC and 25 mg carbopol (F5) showed the highest swelling profile followed by F4 (37.5 mg HPMC and 25 mg carbopol), F10 (62.5 mg HPMC and 25 mg sodium alginate), and F9 (37.5 mg HPMC and 25 mg sodium alginate).

In-vitro drug release study

Figure 4 shows the in-vitro release profile of labetalol hydrochloride from the produced mucoadhesive tablets. Sodium alginate-containing formulations (F6 to F10) showed faster drug release in comparison to carbopol-containing formulations (F1 to F5). F6 formulation showed the fastest release among the ten formulations while F5 showed the lowest release rate. In relation to F6, the release rate decreased by the incorporation of HPMC in F7 and continued to decrease by increasing the proportion of HPMC in F8 through F10. The same pattern was observed in F1 through F5 formulations where F1 showed the fastest release among the carbopol-containing formulations with the release

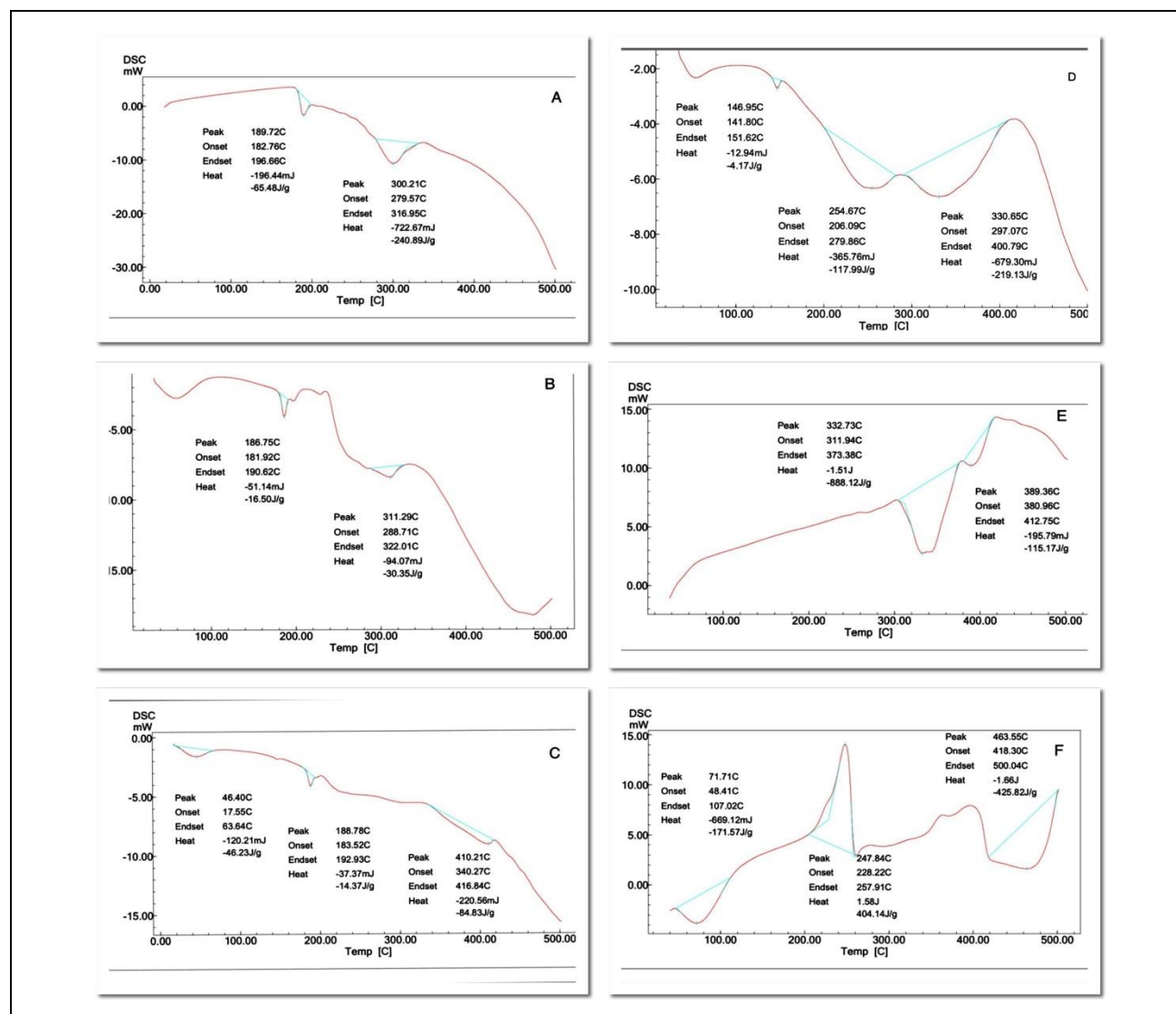


Figure 2. Differential Scanning Calorimetry of (A) labetalol hydrochloride alone, (B) labetalol hydrochloride/ carbopol-934 / Hydroxypropyl methylcellulose (HPMC) mixture, (C) labetalol hydrochloride/ sodium alginate / HPMC, (D) carbopol-934 alone, (E) HPMC alone, and (F) sodium alginate alone.

decreasing through F5. **Table 3** shows the Kinetic parameters of Labetalol hydrochloride release from the produced mucoadhesive buccal tablets. The values of correlation coefficients from the fitted modes suggested that the release of labetalol hydrochloride followed Korsmeyer peipas model. Further exploring of the Korsmeyer peipas model, n values ranged from 0.5 to 1 suggesting non-Fickian drug release behavior.

Ex-vivo evaluation of mucoadhesive buccal tablets

Ex-vivo mucoadhesion time

The adhesion times of carbopol-containing formulations (F1 to F5) were longer than those of sodium

alginate-containing formulations (F6 to F10). Formulations F1 to F5 did not detach from the membrane for the study time (8 hrs) while formulations F6 to F10 were detached in a mean time of 4 hours.

Ex-vivo mucoadhesive strength

Carbopol-containing formulations (F1 to F5) showed stronger mucoadhesion properties than sodium alginate-containing formulations (F6 to F10). The mean \pm SD mucoadhesion force of F1 was 8829 ± 92 dyne while F6 showed mucoadhesion force of 3924 ± 69 dyne. The increment incorporation of HPMC led to better adhesion forces.

Table 2. Physical evaluation of the produced formulations of mucoadhesive buccal tablets

Formulation code	Mean hardness ± SD (kg/cm ²)	Mean surface pH ± SD	Mean weight ± SD (mg)	% Drug content	% Friability
F1	6.67 ± 0.21	4.60 ± 0.23	155.5 ± 4.1	94.6 ± 1.8	0.14 ± 0.02
F2	7.00 ± 0.12	5.34 ± 0.29	156.6 ± 3.6	94.4 ± 1.9	0.14 ± 0.01
F3	7.08 ± 0.13	5.80 ± 0.29	154.4 ± 4.6	95.4 ± 4.6	0.13 ± 0.01
F4	7.22 ± 0.23	6.03 ± 0.50	152.7 ± 3.1	96.6 ± 5.5	0.17 ± 0.03
F5	7.32 ± 0.29	6.70 ± 0.29	153.5 ± 2.6	96.2 ± 3.3	0.13 ± 0.05
F6	4.34 ± 0.42	7.01 ± 0.50	146.9 ± 3.3	94.2 ± 3.4	0.57 ± 0.04
F7	4.36 ± 0.42	7.76 ± 0.25	152.7 ± 3.1	96.4 ± 3.5	0.55 ± 0.03
F8	4.32 ± 0.24	7.83 ± 0.29	153.5 ± 2.6	95.6 ± 4.1	0.49 ± 0.02
F9	4.94 ± 0.26	7.67 ± 0.76	156.6 ± 3.6	96.0 ± 3.1	0.43 ± 0.01
F10	4.94 ± 0.26	8.16 ± 0.29	154.4 ± 4.6	96.2 ± 4.2	0.43 ± 0.02

Ex-vivo transmucosal permeation study

Figure 5 shows the ex-vivo transmucosal permeation of labetalol hydrochloride from the formulated mucoadhesive buccal tablets through the chicken pouch membrane. The permeation of the drug from sodium alginate-containing formulations (F6 to F10) was faster than that from carbopol-containing formulations (F1 to F5). F6 formulation showed the fastest permeation among the ten formulations. The incorporation of HPMC led to decreased permeation in sodium alginate-containing formulations (F6 to F10); with higher proportions of HPMC showing less permeability. This was not the case with carbopol-containing formulations where the plots of F1 to F5 appeared overlapping. The incorporation of increased proportions of HPMC in carbopol-containing formulations seemed to have little influence on the permeation of the drug through the membrane.

The results of the ex-vivo permeation study were correlated with the in-vitro release study results. A strong correlation was detected between in-vivo drug release and ex-vivo transmucosal permeation of labetalol hydrochloride with correlation coefficients of 0.97 for carbopol-containing formulation (F1) and 0.90 for sodium alginate-containing formulation (F6).

In-vivo bioavailability Study

Validation of the HPLC method for labetalol hydrochloride analysis

The limit of detection using 0.75 ml of plasma was 10 ng/ml. The linearity was seen over the concentration range 10-1000 ng/ml. Under these specified conditions the retention times of labetalol hydrochloride was 8.7 minutes.

The calibration curve of labetalol hydrochloride from rabbit plasma give excellent linearity over the concentration range investigated with $R^2=0.9858$. The

linear regression equation of AUC versus concentration (ng/ml) revealed an intercept of 8.965 and a slope of 1.305.

Pharmacokinetic analysis

Figure 6 shows the plasma concentration-time profile of labetalol hydrochloride after oral or buccal administration in rabbits. In general, labetalol hydrochloride plasma concentrations following administration of the mucoadhesive tablets (F1 and F6) were significantly higher than those reached after oral administration of the reference commercial tablets ($p < 0.05$) including the C_{max} . Pharmacokinetic parameters for the formulations F1, F6, and oral commercial tablets are listed in Table 4. Fit was based on last 5 points for oral tablet and F6 formulation while fit was based on last 3 points for F1 formulation.

The mucoadhesive tablets F1 and F6 spent longer times to reach C_{max} in the systemic circulation showing more sustained effect than that of oral one where C_{max} was reached four, two, and one hour after administration of labetalol hydrochloride for mucoadhesive tablet F1, F6, and oral tablet, respectively. In comparison with the reference oral commercial tablet, mucoadhesive tablets F1 and F6 showed relative bioavailability of 2.76 and 1.60, respectively. The observed increase in bioavailability is due to elimination of hepatic pass metabolism as the drug is directly absorbed from buccal mucosa.

The results of ANOVA test showed presence of significant difference between AUC 0-inf., C_{max} and half-life of reference oral tablet and mucoadhesive buccal tablets with p values < 0.0001 .

During the period of the in-vivo study it was observed that F1 and F6 tablets adhered well to the rabbits' buccal mucosa with no signs of redness or irritation at the sites of application.

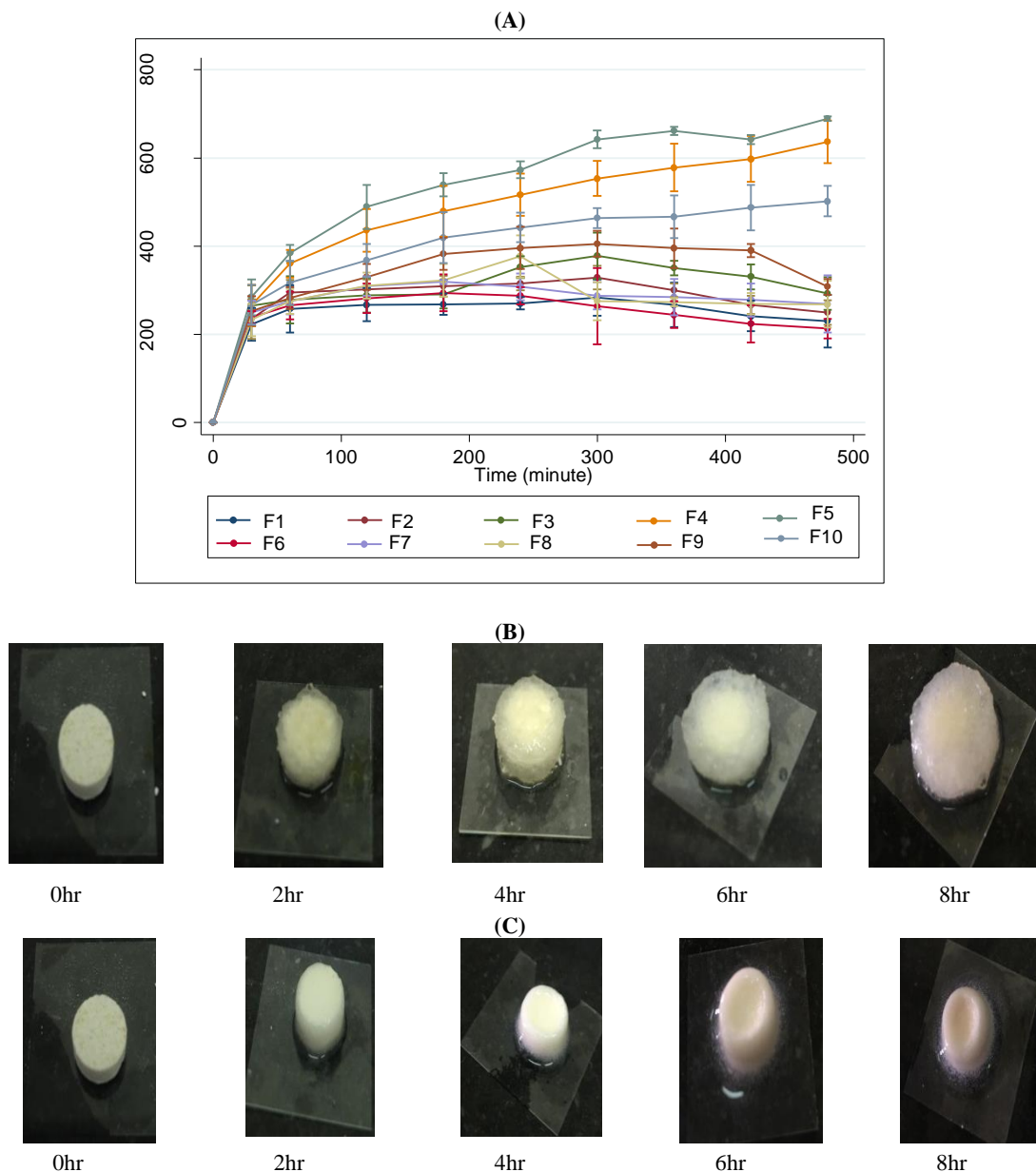


Figure 3. Swelling profiles of the produced mucoadhesive buccal tablets. (A) The swelling index at different time intervals, (B) Pictures of F5 showing the highest swelling, and (C) Pictures of F6 showing the lowest swelling

DISCUSSION

In this study, mucoadhesive buccal tablets containing 25 mg labetalol hydrochloride were formulated and evaluated with the main aim to enhance the systemic bioavailability of the drug. Formulations containing carbopol or sodium alginate plus different proportions of HPMC were investigated. Carbopol-943-containing formulations showed significantly higher hardness, lower percentage

friability, and lower surface pH compared to sodium alginate-containing formulations. Carbopol-943 produced lower surface pH as it is a polyacrylic acid polymer²⁰. Its cross-linked structure contributed to the observed higher hardness and lower friability²¹. The surface pH was suitable for buccal application and no irritation to the buccal mucosa was indicated. This was confirmed in the in-vivo study where the tablets were applied to rabbits' buccal mucosa with no irritation. The mean weight and percentage labetalol hydrochloride

Table 3. Kinetic parameters of Labetalol hydrochloride mucoadhesive buccal tablets

Formulation code	Zero order	First order	Higuchi	Korsmeyer pepas	Korsmeyer pepas (n-value)
F1	0.976	0.919	0.966	0.983	0.648
F2	0.946	0.875	0.986	0.987	0.627
F3	0.974	0.936	0.976	0.995	0.678
F4	0.952	0.889	0.979	0.993	0.679
F5	0.949	0.830	0.814	0.864	0.750
F6	0.983	0.898	0.964	0.997	0.797
F7	0.988	0.911	0.955	0.997	0.816
F8	0.997	0.920	0.936	0.998	0.896
F9	0.984	0.900	0.968	0.998	0.779
F10	0.959	0.983	0.854	0.999	0.825

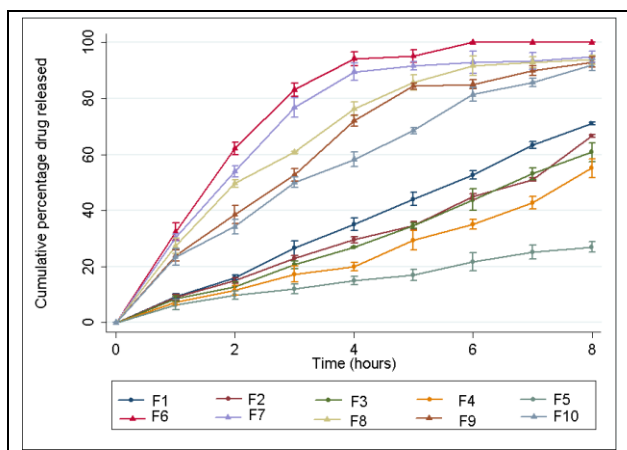


Figure 4. In-vitro release profile of labetalol hydrochloride from the produced bucoadhesive tablets.

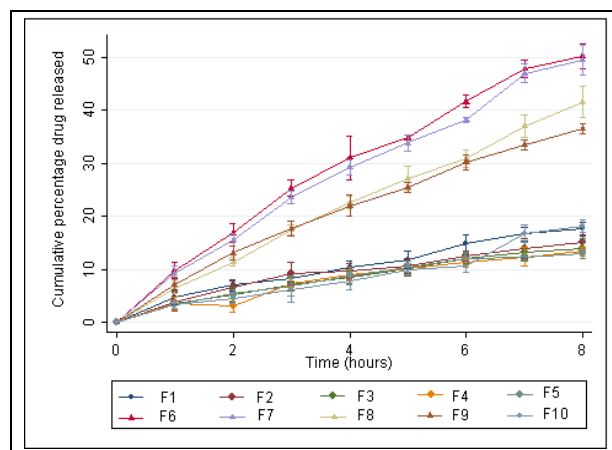


Figure 5. Ex-vivo permeation profile of labetalol hydrochloride from the produced bucoadhesive tablets.

content was uniform and within acceptable ranges as specified by pharmacopeias' recommendations.

The *ex-vivo* mucoadhesion times of formulations containing carbopol-934 were higher than those containing sodium alginate due to the strong mucoadhesive characters of carbopol-934^{20,21}. Similar results were reported by Patel et al., 2007²². Carbopol-containing formulations were associated with higher bioadhesion forces and longer bioadhesive times compared to sodium alginate-containing formulations. This can be explained by the positive charge on carbopol-934 that can produce electrostatic interaction with mucosal membrane²³. The increment in HPMC proportions in the formulations led to increase the bioadhesion force²⁴. This may be explained by the increase of the polymer: drug ratio²⁵.

The swelling profile of the prepared formulations mainly depended on the ratio of HPMC. Formulations with higher proportions of HPMC showed more swelling since HPMC is a hydrophilic water swellable bioadhesive polymer. Similar results were reported in the literature^{24,26}. Formulations containing carbopol showed more swelling properties than those seen in formulations containing sodium alginate; this can be explained by the fact that carbopol can hold larger amount of water into its network.^{9,26} The swelling characters of carbopol is due to the carboxylic group which attain water through hydrogen bonding²⁷. Adequate swelling characters are important to achieve prolonged release of the drug and proper adhesive characters of the mucoadhesive system.

Table 4. Pharmacokinetic parameters following administration of mucoadhesive buccal tablets (F1 and F6) and oral commercial tablets containing labetalol hydrochloride in rabbits

Pharmacokinetic parameters	Commercial oral tablet	Mucoadhesive buccal tablets	
		F6	F1
C_{max} (ng/ml)	81.36	146.49	229.26
T_{max} (hr)	1	2	4
K (hr^{-1})	0.78	0.58	1.09
Half-life time (hr)	0.89	1.19	0.638
AUC 0-6hrs (ng.hr/ml)	221.41	341.35	604.35
AUC 0-infinity (ng.hr/ml)	226.34	361.30	624.27
Relative bioavailability	-----	1.60	2.76

C_{max} : maximum plasma concentration, T_{max} : time taken to reach maximum plasma concentration, K : elimination rate constant, AUC: area under the curve.

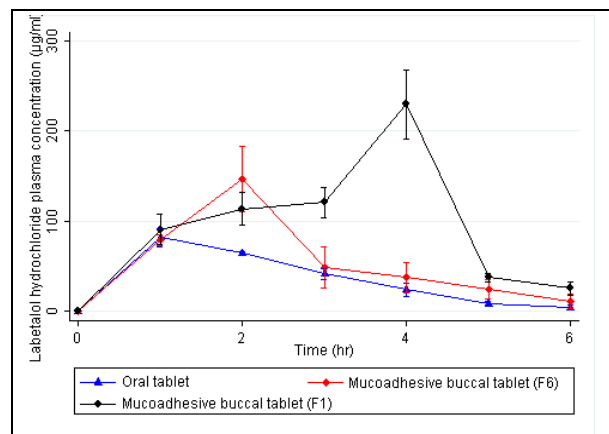


Figure 6. Plasma concentration-time profile of labetalol hydrochloride after oral or buccal administration in rabbits.

Sodium alginate-containing formulations showed the highest in-vitro drug release rates and highest ex-vivo drug permeation rates compared to carbopol-containing formulations. The results were in agreement with different studies in the literature^{22,28,29}. This can be explained by the fact that sodium alginate is hydrophilic in nature resulting in more water absorption and facilitated dissolution and diffusion of the drug³⁰. Carbopol, in the other hand, is water insoluble and hence resulted in decreased drug dissolution rates⁹. In addition, the more swelling properties observed with carbopol compared to sodium alginate may lead to the delay in drug dissolution rates²².

Increasing concentrations of HPMC resulted in decreased in-vitro drug release rate in both carbopol-containing and sodium alginate-containing formulations.

This may be explained by the interaction between HPMC and carbopol or sodium alginate due to the different charges of the polymers. Both carbopol and sodium alginate are anionic in nature while HPMC is non-ionic. The swelling properties of HPMC, increased viscosity, and decreased porosity also contribute to decreased drug release rates³¹⁻³³. Another theory to explain the decreased dissolution rates with increasing the proportions of HPMC may be that the increase in the polymer: drug ratio in general resulting in a more viscous gel like structure that cause decreased diffusion characters^{28,34}. On the other hand, increasing concentrations of HPMC also resulted in decreased ex-vivo drug permeation through chicken pouch membrane in sodium alginate-containing formulations but to a lesser extent in case of carbopol-containing formulations.

The kinetic behavior of labetalol hydrochloride release from mucoadhesive tablets were following a non-Fickian behavior, this was in agreement with previous studies that reported non-Fickian behaviors of tablets containing carbopol^{16,21} and sodium alginate³⁵. This can be explained by the presence of swelling and diffusion which usually do not follow Fickian behaviors³⁶.

The selected mucoadhesive buccal formulations (F1 and F6) showed enhanced systemic bioavailability of labetalol hydrochloride by 2.76 and 1.60 fold, respectively, in comparison to the oral administration. This can be explained by the avoidance of first pass metabolism in the buccal route of administration. Similar results were suggested in the literature where 2-3 fold increase in bioavailability of pentazocine²⁴ and 1.4 fold increase in bioavailability of sumatriptan succinate³⁷ were reported after buccal administration.

The strengths of the current study include the introduction of new promising formulations of

mucoadhesive tablets for the buccal administration of labetalol hydrochloride to offer enhanced bioavailability of the drug. The evaluation of the produced mucoadhesive tablets was performed in multiples levels including the in-vitro evaluation in laboratory apparatuses, ex-vivo evaluation using chicken pouch membranes, and in-vivo evaluation in rabbits.

The current study was limited to the use of three mucoadhesive polymers, namely sodium alginate, carbopol-934, and HPMC; other mucoadhesive polymers such as sodium carboxy methyl cellulose, Xanthan gum, and chitosan may be tested in future studies³⁸. Novel polymers such as thiolated polymers, target-specific, lectin-mediated bioadhesive polymers, and bacterial adhesion could also be investigated³⁹. The techniques used for measuring the mucoadhesion properties included the modified physical balance method for mucoadhesive strength and modified USP disintegration apparatus for mucoadhesion time; other methods including tensile test apparatus / texture analyzer³⁸ or apparatus designed with a digital balance³² could be used.

Formulation and evaluation of other dosage forms, containing labetalol hydrochloride, suggested for buccal mucosal delivery including films/patches, lozenges, and gels can be considered in future studies.

CONCLUSION

In conclusion, formulations containing 17.5% carbopol or 17.5% sodium alginate without HPMC showed the most optimal mucoadhesive properties, in-vitro dissolution rates, and ex-vivo permeation rates. These formulations provided enhanced systemic bioavailability of labetalol hydrochloride by 2.76 and 1.60 folds, respectively, compared to commercial oral tablets of labetalol hydrochloride. Clinical applications of these formulations are recommended.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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