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Original Article

Formulation, Characterization and Evaluation to Establish the Bioavailability of Gastroretentive Mucoadhesive Dosage of Atenolol in Human Subjects with Possible In-Vitro-In-Vivo Correlation

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Abstract

This study was planned to formulate, characterize, and evaluate to establish the bioavailability of gastroretentive mucoadhesive dosage of atenolol in human subjects with possible i*n-vitro-in-vivo* correlation. In this investigation gastroretentive mucoadhesive dosage of Atenolol was formulated using HPMCK_4M, chitosan and Isabgul husk by wet granulation technique. The prepared tablets were subjected to physical evaluation, *in-vitro* drug release, and *in-vivo* X-ray studies, followed by the pharmacokinetic study in human volunteers. All the prepared tablets showed physicochemical properties within the limits and good *in-vitro* mucoadhesion. Formulation F2 was selected based on the *in-vitro* characteristics and *in-vivo* radiographic studies by replacing part of the drug by adding barium sulphate. From the radiographic studies it was found that the F2 could be successfully retained in stomach for more than 6 hours. Pharmacokinetic studies showed a significant improvement in AUC_{0-14}; 6414.93 ± 58.221 ng.h/mL ($p < 0.05$) when compared to reference AUC_{0-14}; 4752.18 ± 76.759 ng.h/mL in healthy human volunteers with good *in vitro-in vivo* correlation. Based on *in-vitro* characteristics and *in-vivo* radiographic studies, F2 was selected as optimized gastroretentive mucoadhesive dosage form with improved bioavailability for better patient compliance and disease management.

Keywords: Atenolol, Bioavailability, *In vitro-in vivo* correlation, Mucoadhesive dosage form, Pharmacokinetic analysis, Radiographic study.

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1. Introduction

The relatively small gastric emptying time (GET) in humans, normally 2-3 h through the major absorption zone i.e stomach and upper part of the intestine can

result in incomplete drug release from the drug delivery system leading to reduced efficacy of the administered dose [1]. Therefore, (Gastro-retentive) dosage form that would be retained in the stomach and release the drug in a controlled manner offers advantages for a variety of important drugs having stability issues or drugs with narrow absorption window in the upper part of gastrointestinal tract (GIT) [2], [3]. Prolonged gastric retention improves bioavailability, reduces drug excess, and improves solubility of drugs suitable for local drug delivery to the stomach and proximal small intestine [4-5].

Atenolol is considered as a good candidate for incorporation into a gastroretentive dosage form due to its better absorption in the upper part of GIT, for prolonged duration so as to achieve maximum absorption and bioavailability [6] with half-life of 6–8 h [7,8]. Thus, it seems that an increase in gastric residence time may increase the extent of absorption and bioavailability of atenolol [9].

Mucoadhesive system is one among the several approaches of oral controlled release dosage forms [10, 11] to formulate a gastroretentive mucoadhesive tablets using mucoadhesive polymers such as chitosan, HPMC K_4M and isabgul husk as no such studies were reported earlier. We developed an optimized formulation for mucoadhesive studies with the simple and novel equipment developed and validated in our lab [12]. The prepared tablets were evaluated for physicochemical properties, *in-vitro* drug release and *ex-vivo* mucoadhesion properties. Based on *in-vitro* drug release and *ex-vivo* mucoadhesion strength, the optimized formulation was subjected to establish the *in-vivo* gastric residence time using X- ray studies. Further, the pharmacokinetics of the optimized tablet was evaluated in human subjects by performing biostudy to establish a meaningful *in vitro- in vivo* correlation.

2. Materials and Methods

2.1. Materials

Atenolol, HPMC K_4M and chitosan was obtained as a gift sample from Dr. Reddy's Laboratories, Hyd, Telagana, India. Isabgul husk (psyllium husk) was procured from Keyur Industries, India. All other reagents used were of analytical grade.

2.2. Preparation of Mucoadhesive of Atenolol Tablets

Accurately weighed quantities of drug along with polymers and microcrystalline cellulose (MCC PH102) were taken in a motor and mixed thoroughly by geometric dilution method. Non aqueous granulation was carried out by using 10 % of PVP K30 in isopropyl alcohol solution. Wet mass was prepared by adding sufficient quantity of PVP solution and passed through 10 mesh. Wet granules were dried at 50 - 60°C for 30 min in hot air oven. Dried granules were

passed through 18 mesh and lubricated with Talc and Magnesium stearate. Final blends was compressed into tablets using 10 mm round flat bevelled punches and corresponding dies on 16 station rotary compression machine (Cemach, India).

2.3. Drug-Excipient Compatibility Studies by Fourier Transform Infrared (FTIR) Spectroscopy

A Fourier Transform – Infrared spectrophotometer was used to study for drug-excipient compatibility. The spectrum of each sample was recorded for the pure drug and optimized formulation in the range 400 to 4000 cm^{-1}.

2.4. Evaluation of Mucoadhesive Strength of Tablets

Measurement of adhesion force was determined by using goat gastric mucus membrane, procured from the local market. The tissue was washed thoroughly with 0.1 N HCl. The membrane was adhered to the base of the equipment (Figure 1) and hydrated using 0.1 N HCl. One side of the tablet was sticked onto the plastic vial cap and the cap was tied to the nylon thread. Other end of the thread was tied to a plastic cup containing counter weight. Thus, the thread was allowed to pass through two pulleys. Now the tablet was placed over the membrane and 50 g weight was placed over the tablet for 15 minutes to induce mucoadhesion. After 15 minutes, an increment of 0.1 g of weight was added to the cup and the counter weight at which the tablet detaches from the membrane was determined. From the mucoadhesive strength, the force of adhesion was calculated using the formula as given below [12, 13].

$$f_1 = \frac{\sum_{j-1}^{n} |R_j - T_j|}{\sum_{j-1}^{n} R_j} X\ 100$$

2.4.1. Ex-vivo Mucoadhesion Time

The *ex-vivo* mucoadhesion time was examined by placing a tablet over excised goat mucosa for 5 minutes after being secured on a glass slide. The slide containing tablet was immersed in the USP paddle type dissolution apparatus containing 900 mL of 0.1 N HCl and the temperature was maintained at 37 ± 0.5^0C. A distance of 2.5 cm was adjusted for the paddle of the dissolution apparatus from the tablet which was rotated at 50 rpm. The time taken by tablet to detach from the membrane was recorded in hours.

2.5. Physico-Chemical Parameters of Compressed Mucoadhesive Tablets of Atenolol

The physico-chemical parameters of compressed mucoadhesive tablets were evaluated for weight variation, thickness, hardness, friability and drug content as per the USP pharmacopeial norms.

2.6. Swelling Studies

2.6.1. Determination of Swelling Index of Tablets

Radial swelling of the tablet was monitored by immersing the tablet in beaker containing

250 mL of 0.1N HCl dissolution medium at room temperature. An increase in the tablet diameter was estimated for the period of 24 hours. The same was measured in at least two different places perpendicular to each other and their mean value was taken. Swelling index (SI), expressed as percent, was calculated as per the following equation:

SI= {[dt-di]/di} x 100

Where,

Dt= tablet diameter at time t

Di=b Initial diameter of tablet

2.7. In vitro Drug Release Studies

The USP Type II dissolution apparatus was used for testing *in-vitro* drug release for the mucoadhesive tablets by adhering the tablet onto a glass slide using hard paraffin. The glass slide was then placed in the dissolution medium of 900 mL 01.N HCl with the rotational speed of 50 rpm at 37 ± 0.1ºC. Five mL of samples at time intervals of 0.5, 1, 2, 3, 4, 6, 8, 10, 12 h were withdrawn and again replenished with equal volume of fresh media. The drug content of atenolol from the samples was determined at 274 nm.

2.8. In-Vivo Gastric Residence Time Determination (X-Ray Studies)

The in-vivo x-ray studies were approved by the Institutional Ethical Committee with approval No. IHEC/VGOPC/059/2015. The mucoadhesive tablet was administered to the healthy human volunteers weighing 50 -70 kg (n = 3) with an age range between 20 - 35 years. In this X-ray study, the optimized formulation was developed by replacing 60 mg of atenolol with X-ray grade barium sulfate as a radio-opaque substance thus, keeping all other ingredients constant. The optimized formulation thus developed was evaluated for mucoadhesive properties as stated above (data not shown). The *in-vivo* gastric residence time determination was carried out under fed conditions. Subsequent X- Ray determination was carried out at 15 min, 2, 4, 6 and 8 hours respectively [14, 16]. The same X-ray procedure was also performed after the stability period.

2.9. Stability Studies

To assess the drug and formulation stability, accelerated stability studies were done according to ICH and WHO guidelines [17, 18]. Optimized formulation was kept in the humidity chamber (Pooja labs, India) maintained at 40 °C and 75% Relative Humidity for 6 months. At the end of studies, samples were analyzed for physicochemical parameters. For the comparison of release profiles of initial and stability samples, "difference factor" f_1 and "similarity factor" f_2, were calculated. The difference factor (f_1) measures the percent error between the two curves over all time points and was calculated as follows:

$$f_1 = \frac{\sum_{j=1}^{n} |R_j - T_j|}{\sum_{j=1}^{n} R_j} X\,100$$

Where n is the number of sampling points, Rj and Tj are the percent dissolved of the

reference and test products at each time point j. The two release profiles are considered to be similar, if f_1 value is lower than 15 (between 0 and 15). The similarity factor (f_2) is a logarithmic transformation of the sum of squared error of differences between the test Tj and the reference products Rj over all time points. It was calculated using the following equation:

$$f_2 = 50 \log \{[1 + (1/n)^{-0.5} \sum^{n}_{j-1} w_j |R_j - T_j|^2]\} \text{ X } 100$$

Where *wj* is an optional weight factor and other terms are as defined earlier. The two dissolution profiles are considered to be similar, if f_2 value is more than 50 (between 50 and 100) [19].

2.10. Bioavailability Study of Atenolol GRDDS in Healthy Human Volunteers

This work describes the bioavailability study of atenolol GRDDS compared against standard reference product. Before conducting the bioavailability study, a detailed report on the study was submitted to Institutional Human Ethical Committee and study approval was obtained (IHEC/VGOPC/059/2015).

2.10.1. Subjects

Twelve male volunteers, aged between 21 – 35 years and weighing between 50 – 75 kg participated in the study. The subjects were non-smokers and ensured that they were not taking any kind of medication before and during the study. All the volunteers were found healthy after thorough clinical examinations and each volunteer was explained about the study design, drug pharmacokinetics and its effects along with the probable adverse effects or side effects. The informed consent was obtained from each of them prior to study.

2.10.2. Study Products

The test product was optimized study formulation (F2) containing atenolol 100 mg (Table 1). The Aten 100 mg (manufacturing company-Zydus cadilla) immediate release tablets were purchased commercially as a reference product.

2.10.3 Study Design

This study was performed in a two-way crossover design with a washout period of 2 weeks between two phases. All the volunteers were subjected to overnight fasting before drug administration with no prior medication history. The subjects were randomly divided into two groups. The optimized GRDDS containing 100 mg of Atenolol was administered to one group and Aten 100 mg was administered to another group with a 250 mL of water after taking a light and standardized breakfast as fed conditions. Standardized lunch was provided after 6 hrs after consultation with qualified nutritionist. Venous blood samples (4 mL) were withdrawn into the clot activator tubes at 0 hour and at 1, 2, 3, 4, 6, 8, 10, 12 and 14 hours after drug administration. The blood samples were centrifuged at 4000 rpm and serum was transferred immediately to the screw-cap tubes and stored at -20°C until analysis.

2.10.4. Apparatus and Analytical Conditions

A LC-20 AD Shimadzu with programmable SPD-20A prominence UV/ Vis detector was used for drug serum analysis. The HPLC mobile phase was composed of phosphate buffer pH 6.8- methanol (75:25, v/v) containing 0.06% of ortho phosphoric acid (OPA). Separation was achieved using a Hibar- Lichrospher RP- 18 column (5µm, 4.6 x 250mm i.d.) at a flow rate of 1mL/ min. The eluent was monitored by UV/Vis detector at 225 nm.

2.10.4.1. Construction of Calibration Curve of Atenolol in Human Serum

A 0.5 mL of serum was taken in a 1.5 mL eppendrop tube and 100µL of various concentrations of atenolol solution was added in order to achieve 50, 100, 200, 300, 400, 500, 1000, 1500, 2000, 2500 and 3000 ng/mL solutions. To the above solutions 100 µL of hydrochlorthiazide was added as internal standard (500 ng/mL) and made upto 1mL with methanol in a 1.5 mL eppendrop tube, mixed for 2 minutes on a cyclomixer for protein precipitation and after protein precipitation, centrifugation was carried out for 20 minutes at 12000 rpm using microcentrifuge. A 20 µL aliquot of supernatant was injected into the column. A calibration curve was plotted by taking the ratio of AUC of drug / IS at various concentrations.

2.10.4.2. Analytical Method Validation

Calibration curve (n = 3) was obtained as described above and methods were validated prior to assay for linearity, precision, accuracy, limit of detection and quantification according to US Pharmacopoeia [20] and International Conference on Harmonization (ICH) guideline [21]. Linearity of the standard curve was $r^2 > 0.998$. The intra-day and inter-day precision of the assay was assessed by calculating the relative standard deviation (RSD). The drug recovery was determined from drug-free serum spiked with known amounts of atenolol (50, 500 and 3000 ng /mL).

2.11. Pharmacokinetic Data Analysis

2.11.1. Pharmacokinetic Parameters

Atenolol plasma concentration-time data were analyzed for each subject using non-compartmental method. Basic pharmacokinetic parameters required for the comparison of bioavailability, such as peak serum concentration (C max), time to reach the peak serum concentration (t max), area under the serum concentration time curve (AUC), elimination half-life ($t_{½}$) and mean residence time (MRT) for the drug under observation were obtained in each subject from plasma concentration versus time profiles using KINETICA software (version 5).

The % increase in the AUC was calculated as per the formula.

$$\% \text{ increase in } AUC_{0\text{-}t} = \frac{|AUC_{test} - AUC_{reference}}{AUC_{test}} \times 100$$

The extent of improvement of bioavailability was calculated as per the formula.

$$\text{Extent of increase in } AUC_{0\text{-}t} = \frac{AUC_{test}}{AUC_{reference}}$$

2.12. In Vitro - In Vivo Correlation between In-Vitro Percent of Drug Release and Mean Area under the Curve of Test Product

The cumulative percentage of atenolol released in-vitro from the optimized GRDDS was compared against the extent of absorption i.e., mean cumulative AUC values of volunteers of test product for a possible *in vitro – in vivo* correlation.

2.13. Statistical Analysis

All the data was statistically analyzed using Graphpad prism software (version 6). Paired t-test was used for comparison of pharmacokinetic parameters. A value of $p < 0.05$ was considered to be significant for all results.

3. Results and Discussion

This research work was carried out with an intention to develop an optimized mucoadhesive GRDDS of Atenolol. Earlier, several investigators worked on Atenolol considering it as a model drug for GRDDS but very little work was carried out in the field of mucoadhesive systems. So an attempt has been made to develop an optimized mucoadhesive system for Atenolol.

3.1. Calibration Curve of Atenolol

An UV- spectrophotometric method was used for determination of atenolol. The standard graph of atenolol was prepared in 0.1 N HCl (pH 1.2) at 274 nm. Calibration curve was plotted and found to be linear from 0 -14 µg/mL ($y = 0.47x + 0.003$, $r^2=0.999$).

3.2. Fourier Transform Infrared (FT-IR) Spectroscopy

The Infrared (FT-IR) spectra indicated no interaction for the major functional groups, without any major shifts of peaks.

3.3. Evaluation of Physico-Chemical Parameters of Mucoadhesive Tablets of Atenolol

All the prepared batches of tablets tested for physico-chemical parameters like hardness, thickness, weight variation, friability and drug content were found to be within the pharmacopeial limits of USP.

3.4. Effect of Bioadhesive Polymers on Mucoadhesive Strength, Mucoadhesive Force and Mucoadhesive Time

In our study, the mucoadhesion of dosage form to gastric mucosal membrane to determine the mucoadhesive property was carried out using a novel and simple method developed within the lab. The developed method was also validated for its reproducibility. Randip *et al*. developed mucoadhesive gastroretentive bilayer tablets of Pantoprazole using synthetic mucoadhesive polymers Carbopol 934P, HPMC K100M, and Sodium CMC with ethyl cellulose as backing layer and determined the mucoadhesive properties by *in vitro* method [22, 23]. Earlier S.K Singh et.al [13] and several others utilized

a modified physical balance to determine the mucoadhesive strength, but our method of determination of mucoadhesive strength was more simple and reliable to determine the mucoadhesive strength [24].

All the formulations were tested for mucoadhesive strength; mucoadhesive force and mucoadhesion time (Table No.2). All the batches showed good *ex- vivo* adhesive properties. The mucoadhesive strength and mucoadhesive force was found to be in the range of 16.2- 22.56 g and 1.59 N. Total mucoadhesion time was observed for more than 10 hours. Formulation F2 containing chitosan and HPMC K_4M in combination showed higher mucoadhesion than others and was considered for optimization.

3.5. Swelling Studies

The swelling index indicated good water uptake and hydration of mucoadhesive formulations and the optimized formulation F2 showed more than three times increase in initial diameter by the end of 24 hours in 0.1N HCl. The dosage form gradually increased in radial diameter with respect to time and 310% swelling index was observed for 24 hours (Figure 2).

3.6. In-Vitro Drug Release and Mathematical Modeling of Dissolution Profiles

Several studies reported the mucoadhesive controlled drug release using chitosan [25], HPMC K_4M and Carbopol 934 [26 , 27] but our study got desired drug release, better mucoadhesion and swelling when chitosan, HPMC K_4M and isabgul husk were used in combination. Therefore polymers in the combination were utilized to obtain optimum mucoadhesion together with constant drug release.

The *in-vitro* drug release studies for the formulations F1 to F9 were studied. The F2 and F8 released more than 90% up to 12 hours and remaining formulations were unable to release and sustain the drug completely for more than 12 hours which may be due to improper wetting of the matrix as high concentration of polymer was utilized in order to achieve good mucoadhesion (Figure 3, Figure 4, and Figure 5).

The correlation of coefficient (r^2) values of all the mucoadhesive tablets for zero order release kinetics was found to be higher than that of first order release kinetics indicating that drug release followed zero order kinetics. The correlation coefficient values of Higuchi's square root time model for all the formulations were found to be more than 0.90 indicating that drug release followed by diffusion mechanism. The diffusional exponent values (n) were more than 0.45 for all the formulations except F6 and F9 (Table 3) indicating that the release from matrices followed non-fickian diffusion (diffusion and erosion controlled). F6 and F9 followed fickian diffusion (diffusion controlled).

Based on i*n-vitro* drug release and mucoadhesive properties (Table 2 and Table 3), F2 formulation was selected as optimized formulation over F8. Further, it was subjected to stability studies, swelling studies, *in-vivo* radiographic studies and for assessment of bioavailability in human volunteers.

3.7. X-Ray Studies

Previously Doodipala N, Katakam V.K [14-16] and several others reported the radiographic studies in human volunteers for floating GRDDS with a gastric retention time of 5-6 hours. Based on earlier reports we conducted *in-vivo* radiographic studies [28, 29].The radiographic images taken at different time intervals after oral administration of the tablet were observed in human stomach after 15 minutes followed by 2, 4, 6 and 8 h respectively. No significant changes were detected with tablet remaining in its position. This evidenced that the tablets were adhered to the gastric mucosa and remained in the stomach for more than 6 h. The next radiographic picture at 8 h showed the dosage form nearly in the region of duodenum thus suggesting that the dosage form detached from the gastric mucosa and followed its normal gastrointestinal transit behavior (Figure 6). Hence the study proved that the formulated optimized dosage form was successfully retained in the stomach for more than 6 hours.

3.8. Stability Studies

The results of the accelerated stability studies indicated a good physical stability without any variations in assay and mucoadhesive properties. The values of similarity factor and difference factor for the *in-vitro* drug release profiles of optimized formulation during the storage period indicated good stability. The analysis of the dissolution data of optimized formulation F2 after storage at 40°C±5°C/75% ±5% RH for 6 months showed no significant changes indicating the two dissolution profiles are considered to be similar (f_2 value was more than 50 i.e 91.01 at 3rd month and 82.60 at 6th month and f_1 value less than 15 i.e 2.85 at 3rd month and 4.34 at 6th month) with good similarity between dissolution profiles during the stability period (Figure 7). After the stability period, the optimized formulation was tested for its *in vivo* residence time by X-ray studies and it was found that the dosage form does not altered its position when the radiographic images taken at different time intervals such as 15 minutes, 2, 4 and 6 h. This proved that the formulation is stable and showing good mucoadhesive property.

3.9. In Vivo Bioavailability Study of Atenolol GRDDS in Healthy Human Volunteers

3.9.1. Calibration Curve of Atenolol in Human Serum

The well resolved peaks of atenolol and hydrochlorothiazide (IS) were obtained at 4.9 for Atenolol and 5.9 minutes for hydrochlorothiazide (Figure 9 and Figure 10). The blank serum after protein precipitation consistently had no interference with the drug and IS peaks (Figure 8). The ratio of area under the curve (AUC) of Atenolol to IS was linear from 50 to 3000 ng/mL (y= 0.0012x, r^2= 0.9980). The drug recovery of atenolol (50, 500 and 3000 ng /mL) was less than 5%. The lower limit of detection (LLOD) and lower limit of quantification (LLOQ) were 20 ng /mL and 50 ng /mL respectively. The intra- and inter-day % RSD was within 15%. The

accuracy of atenolol ranged between 100.24 and 101.66%.

Hye Sun Gwak *et al*. and several other authors reported the pharmacokinetics of the immediate release Atenolol tablets [30]. Similarly Mazumdar *et al*. also reported the pharmacokinetics of floating single units of Atenolol in rabbit model [9]. Our study planned to compare a mucoadhesive GRDDS with an immediate release marketed formulation (Aten 100 mg) to find out any possibility of improvement in bioavailability.

Fig.11 describes the mean plasma concentration – time profiles for both test and standard tablets. The two curves are quite comparable and significant in drug concentrations of the test formulation. The mean peak plasma concentration, C_{max} which describes the rate of drug absorption was lower for test when compared to reference with a delay in achieving the peak plasma concentration. The mean AUC that describes the extent of drug absorption was higher for test when compared to reference. The $t_{1/2}$ and MRT suggests the residence period of drug molecules and describe elimination characteristics of the drug was prolonged in the test product when compared to standard suggesting the retaining capability of the dosage form and improving the bioavailability. The mean values of C_{max}, T_{max}, AUC, $t_{1/2}$, and MRT are listed in Table 4.

The results from statistical analysis by student paired t- test at $p < 0.05$ level for test product was found to be significant when compared to that of reference for all the pharmacokinetic parameters indicating that there is a significant difference between the test and reference product. The % increase in AUC_{0-t} was calculated and the improvement was found to be 25.92% with a 1.349 fold improvement in extent of bioavailability. The percent increase and extent of bioavailability suggest that there is a marked improvement in bioavailability of test product when compared to reference standard.

3.10. In Vitro and In Vivo Correlation

To establish *in vitro – in vivo* correlation between cumulative percent drug release versus cumulative AUC. A best fit line was drawn to get level A correlation with r^2 value of 0.957. This shows controlled manner drug release in proportion to its absoption through the lower gastrointestinal tract (Figure 12). The *in vitro- in vivo* correlation suggests that as the cumulative percent of drug released in control manner, was being getting absorbed through the lower gastrointestinal tract.

4. Conclusion

From this investigation it is concluded that the adopted method successfully produced uniform and reproducible mucoadhesive tablets of Atenolol with chitosan, $HPMCK_4M$ and Isabgul husk polymers showing non fickian zero order drug release for the optimized formulation F2. *In vivo* radiographic studies revealed that it could be retained in stomach for more than 6 hours. From the biostudy, it is concluded that the formulated optimized formulation showed an improved bioavailability compared to reference with good *in vitro- in vivo* correlation.

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Figures:

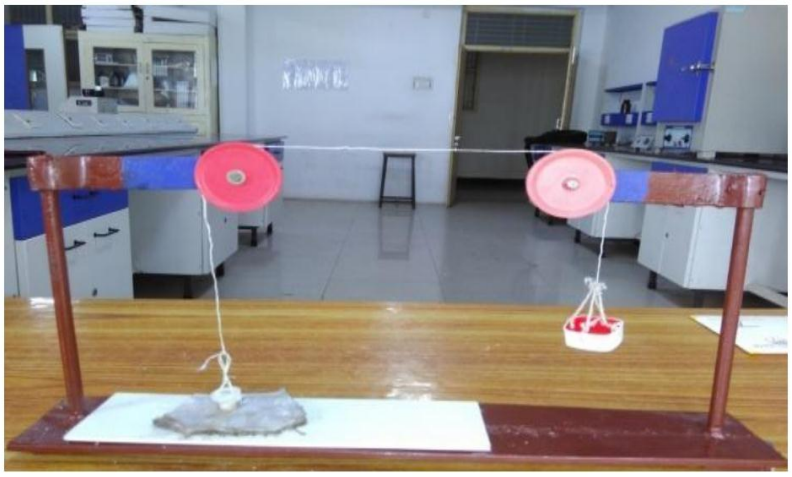

Figure 1. Method developed for determining mucoadhesive strength.

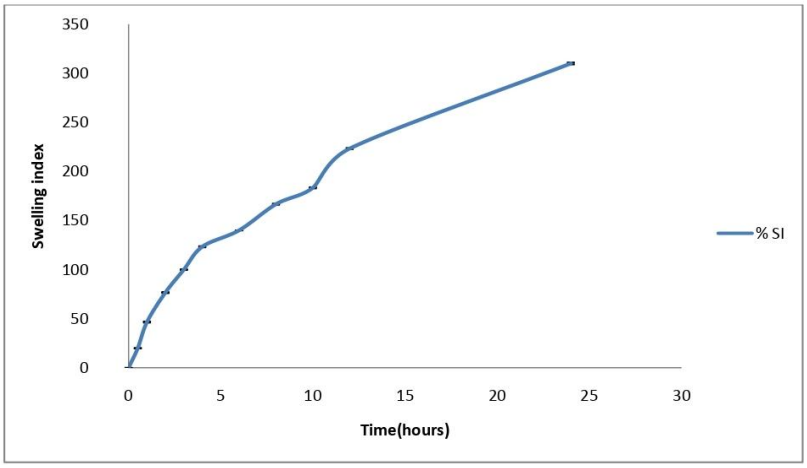

Figure 2. Swelling index of optimized formulation FM2 containing Chitosan and HPMC K_4M.

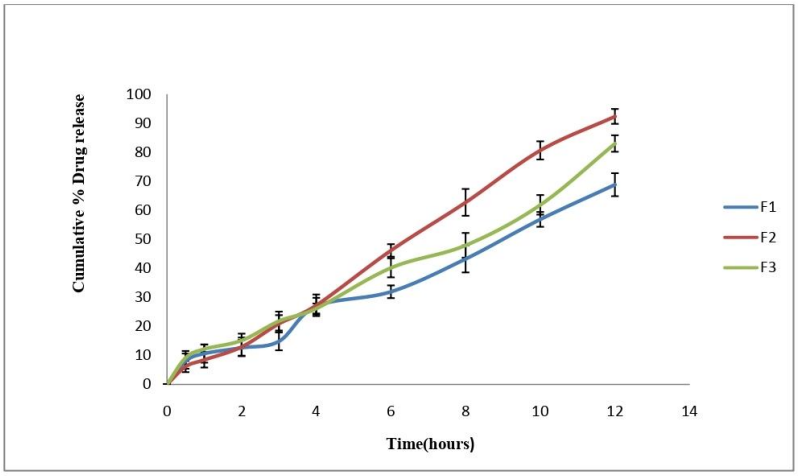

Figure 3. Dissolution profile of formulations prepared with chitosan and HPMC K_4M polymers.

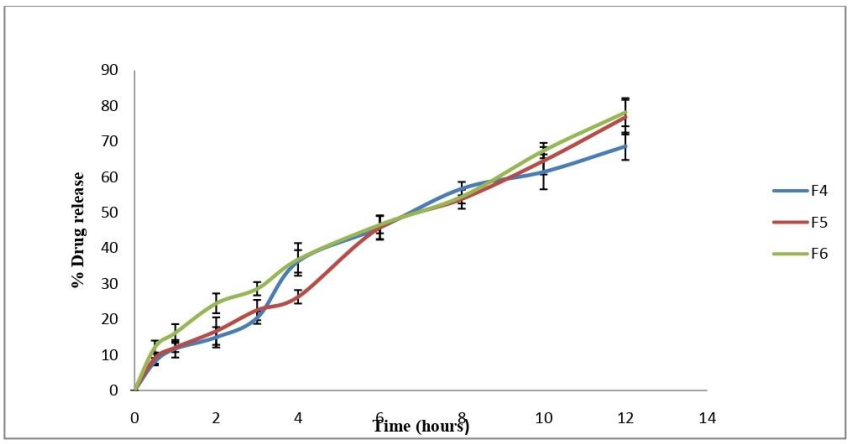

Figure 4. Dissolution profile of formulations prepared with Isabgul husk and HPMC K_4M polymers.

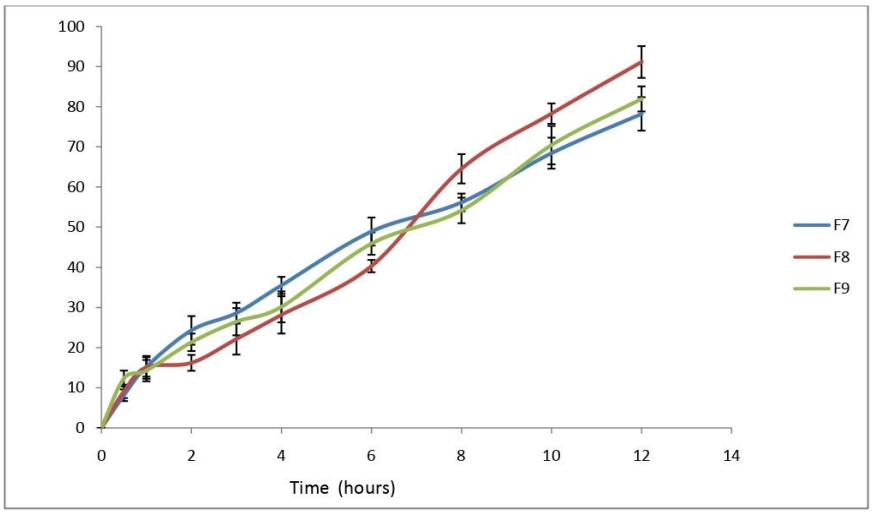

Figure 5. Dissolution profile of formulations prepared with Chitosan, Isabgul husk and HPMC K_4M polymers.

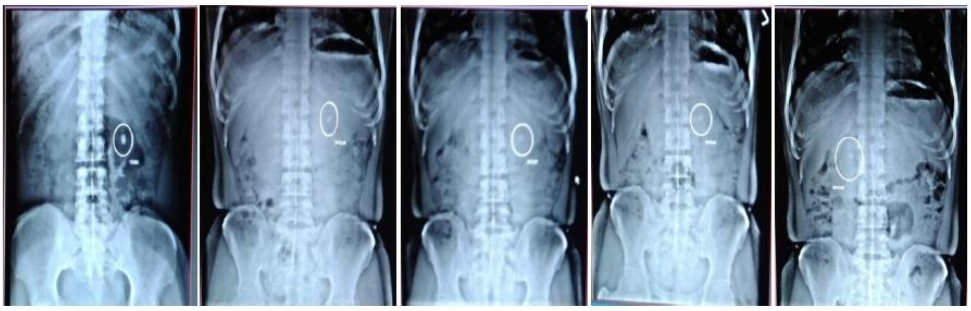

Figure 6. Radiographic pictures of human volunteers taken at different time intervals.

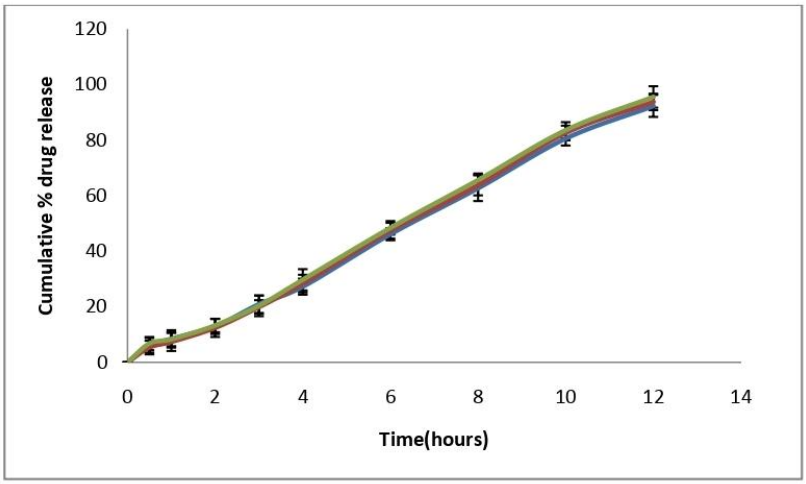

Figure 7. Stability studies showing cumulative percentage drug release of optimized formulation F2 at 0,3rd and 6th month.

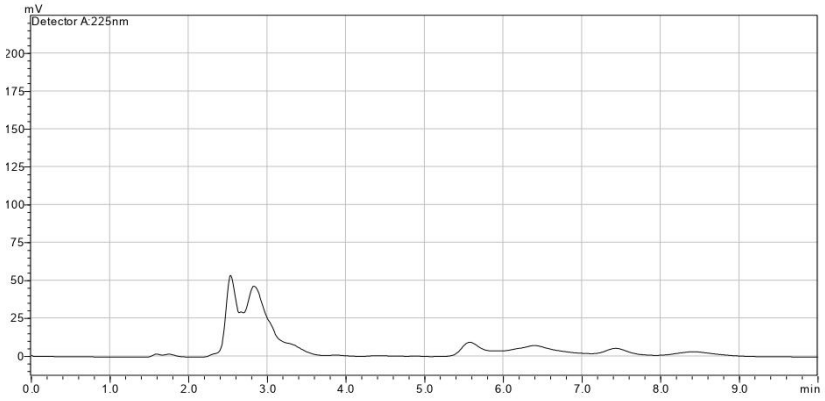

Figure 8. Chromatogram of blank serum.

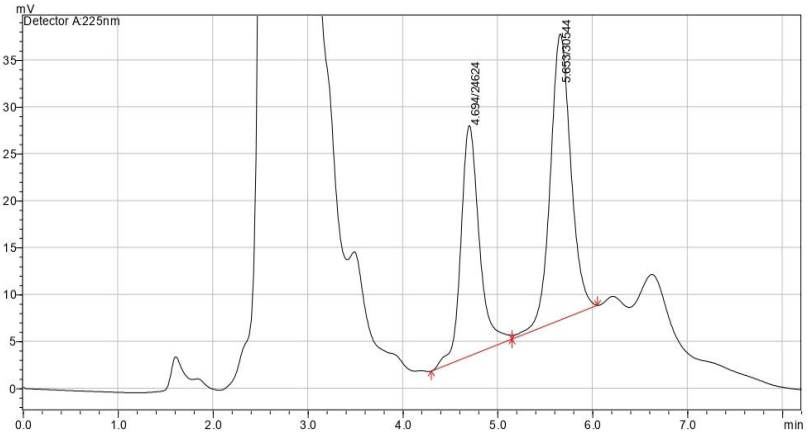

Figure 9. Chromatogram of test (3rd h).

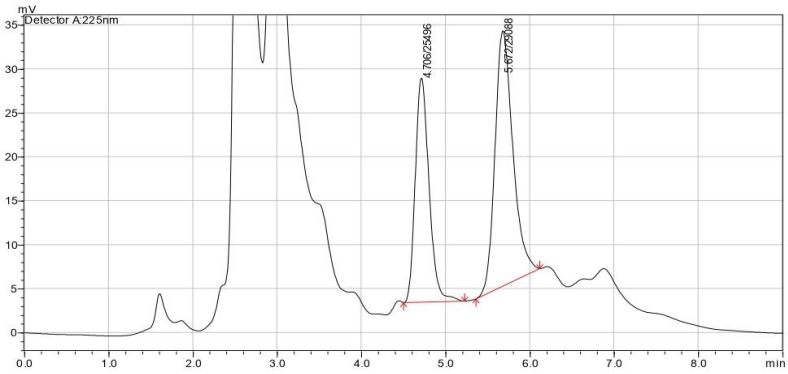

Figure 10. Chromatogram of Standard (3rd h).

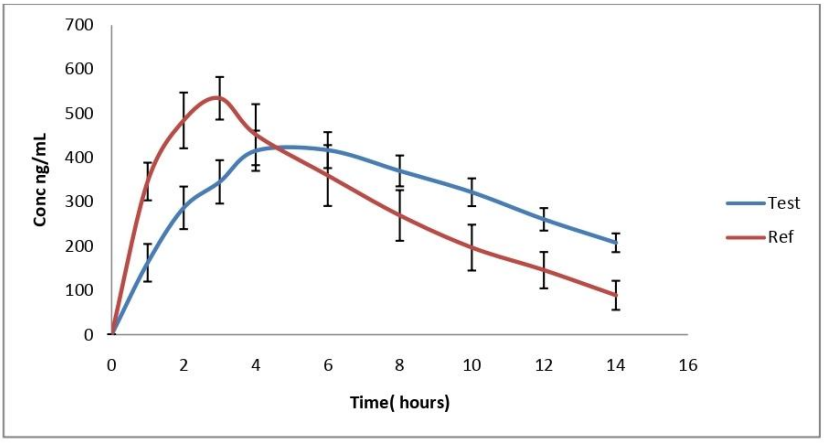

Figure 11. Pharmacokinetic profiles of mean plasma concentrations vs time of atenolol for test and reference sample.

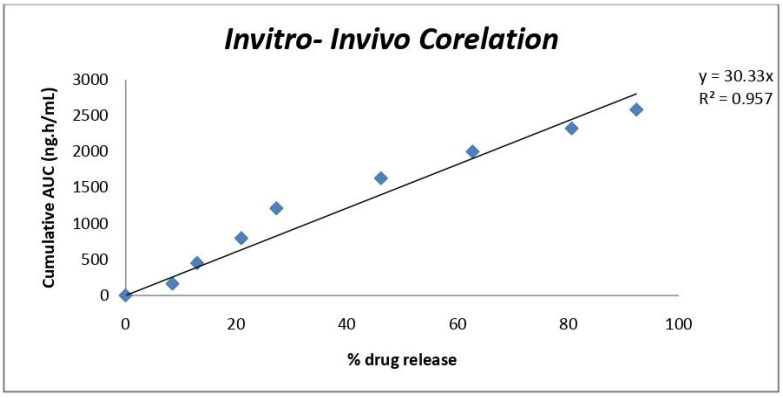

Figure 12. *In vivo- In vitro* correlation of optimized atenolol mucoadhesive tablet.

Tables:

Table 1. Tablet composition of mucoadhesive tablets of Atenolol.

Formulation code	Drug (Atenolol) (mg)	Chitosan (mg)	$HPMCK_4M$ (mg)	Isabgul husk (mg)	MCC PH 102 (mg)	Talc (mg)	Magnesium stearate (mg)
F1	100	100	200	_	85	10	5
F2	100	200	100	_	85	10	5
F3	100	150	150	_	85	10	5
F4	100	-	200	100	85	10	5
F5	100	_	100	200	85	10	5
F6	100	_	150	150	85	10	5
F7	100	_	300		85	10	5
F8	100	300			85	10	5
F9	100	_		300	85	10	5

Each tablet weight is 500 mg, with a hardness in-between 4 – 5 $kg/cm^{2.}$

Table 2. Mucoadhesive properties of prepared Atenolol mucoadhesive tablets.

Formulation code	Mucoadhesive strength (g) Mean ± S.D (n= 5)	Mucoadhesive force (N)	Mucoadhesive time (hours)
F1	21.37 ± 0.29	2.09	> 12
F2	22.56 ± 0.42	2.21	> 12
F3	19.64 ± 0.26	1.92	> 12
F4	18.92 ± 0.35	1.82	> 12
F5	16.5 ± 0.52	1.62	> 12
F6	16.2 ± 0.37	1.59	> 10
F7	18.42 ± 0.32	1.80	> 12
F8	19.64 ± 0.25	1.92	> 12
F9	16.52 ± 0.35	1.62	> 10

Table.3. Regression coefficient (R^2) values of mucoadhesive tablets for different kinetic models.

Formulation code	R^2				Peppas (n)
	Zero	First	Higuchi	Korsmeyer and Peppas	
F1	0.993	0.977	0.954	0.572	0.452
F2	0.997	0.951	0.952	0.698	0.653
F3	0.992	0.941	0.953	0.615	0.464

F4	0.981	0.995	0.979	0.639	0.496
F5	0.995	0.985	0.971	0.632	0.476
F6	0.988	0.987	0.988	0.633	0.399
F7	0.989	0.992	0.989	0.669	0.480
F8	**0.**993	0.948	0.949	0.654	0.507
F9	0.994	0.974	0.973	0.620	0.420

Table 4. Pharmacokinetic parameters obtained for reference and test product after oral administration.

Volunteer	**C_{max} (ng/mL)**		**T_{max} (h)**		**AUC_{0-14} ng.h/mL**		**$AUC_{0-infinity}$ ng.h/mL**		**$t_{1/2}$ (h)**		**MRT (h)**	
	R	**T**	**R**	**T**	**R**	**T**	**R**	**T**	**R**	**T**	**R**	**T**
Mean n= 12	555.21	439.22	2.66	4.83	4752.18	6414.93	5346.95	8471.57	4.37	6.78	7.6	12.43
SD	34.07	34.57	0.49	1.02	76.75	58.22	110.43	102.25	0.65	1.15	1.05	1.36

R= reference standard product, T= test product, $t_{1/2}$= elimination half-life, MRT= Mean residence time.