RESEARCH ARTICLE JPAR 2018

Journal of Pharmaceutical Advanced Research

(An International Multidisciplinary Peer Review Open Access monthly Journal)

Available online at: www.jparonline.com

Atenolol Buccal Patches: *In vitro* – *Ex vivo* studies

Surya Narayan Ratha Adhikari*, Satyabrata Panda

Department of Pharmaceutics, Seemanta Institute of Pharmaceutical Sciences, Mayurbhanj, Odisha, India.

Received: 28.08.2018 Revised: 29.08.2018 Accepted: 30.08.2018 Published: 31.08.2018

ABSTRACT: Background: The buccal drug delivery is an excellent route for increasing bioavailability and preventing first pass metabolism with decreasing dose and dosing frequency. **Aim:** The present investigation focussed on formulating and evaluating atenolol buccal patches. **Methods:** The buccal pathes were prepared by solvent casting method using film forming polymers like fenugreek seed mucilage, Hydroxy Propyl Methyl Cellulose (HPMC) and sodium alginate. The prepared buccal pathes were evaluated for various physico mechanical properties like thickness, weight variation, folding endurance, drug content, surface pH, moisture content, moisture uptake and surface morphology study. The *in vitro* release study was carried out in Franz diffusion cell using commercial semi permeable membrane. The *ex vivo* permeation study was carried out using goat buccal mucosa. The stability study of the optimized formulation F3 was performed as per ICH guidelines. **Results:** The evaluation data reaveled satisfactory physicochemical characteristics. The drug content was found to be uniform in the range of 98.94 ± 0.12 (F4) to 99.41 ± 0.13 % (F1). The buccal patch F3 showed highest drug relase (81.3 %) and permeation (73.2 %). Both *in vitro* drug release and *ex vivo* drug permeation studies showed sustained drug release profile. The optimized patch was found to be stable with good surface morphology characteric. **Conlusion:** It was concluded that the atenolol bucal patch formulation, F3 containing fenugreek seed mucilage (125 mg) and HPMC (125 mg) as sustained release polymers, was found to be best optimized formulation which might be used for safe management of hypertension.

Corresponding author*
Mr. Surya Narayan Rath Adhikari
Asst. Professor
Seemanta Institute of Pharmaceutical Sciences, Mayurbhanj, Odisha, India.
Mail ID. snarayan.rath@gmail.com
Tel No. +91-9861285212.

Keywords: Buccal, Pathes, Atenolol, Hypertension, *ex vivo* permeation and fenugreek seed mucilage.

INTRODUCTIONS:

Amongest various routes of drug delivery buccal route found increased significance due to improved patient compliance, over coming fast pass metabolism, increasing bioavailability with decreasing dose and dosing frequency [1,2]. Atenolol chemically is a benzene acetamide. It is soluble in water (26.5 mg/ml at 37 °C). Its partition coefficient value is 0.23. Atenolol is incompletely absorbed (about 50 %). It's T_{max}, is 2 to 4 h. The elimination half-life of atenolol is 6 to 7 h. Its bioavailability and plasma protein binding is 55 and 15 %. Atenolol possesses side effects like chronic fatigue,

sleep disturbance, insomnia, nightmares, depression, sexual dysfunction and impotence [3-5]. The aim of the present investigation was to develop and evaluate buccal patches containing Atenolol and different film forming polymers like fenugreek seed mucilage, HPMC and sodium alginate.

MATERIALS and Methods:

Materials:

Atenolol was obtained from M/S. P.D.I.L, India. Fenugreek (*Trigonella foenum-graecum* L.) seed mucilage was isolated from the raw fenugreek seeds. HPMC K4M and ethyl cellulose were obtained from Matrix Laboratories, India. Propylene glycol was purchased from Burgoyne Burbides and Co., Mumbai, India. Sodium alginate was obtained from HiMedia Laboratories Pvt.Ltd. Dibutyl phthalate was obtained from Ranbaxy Laboratories, India. All other reagents used were of analytical grade and procured from Authorised dealer.

Preparation of atenolol buccal patches:

The mucoadhesive buccal patches were prepared by solvent casting technique using different ratios of fenugreek seed mucilage, HPMC and sodium alginate as film forming polymers. The buccal patches containing atenolol (~25 mg/cm^2 patches) in the 38 cm^2 petridish. Propylene glycol was incorporated in the concentration of 10 % of dry weight of polymers as a plasticizer. Dimethyl sulfoxide (DMSO) in 1 % and 5 % dry weight of polymers was incorporated as penetration enhancer. Backing membrane was prepared by pouring and evaporating 6 % ethyl cellulose in 65:35 ratio of acetone: isopropyl alcohol and 15 % w/w of dibutyl phthalate of the polymer in room termperature for 12 h. The matrix was prepared by pouring 25 ml of the homogeneous solution on the backing membrane in a petridish and dried at 40 °C in the incubator. After 24 h the patch was removed from petridish, before removing patch was dried at 37 ^{0}C for 1 h. The dry patches were placed in desiccators until use [6,7].

Characterization of atenolol buccal patches:

Measurement of average weight and thickness:

Three buccal patches from each batch, as a whole (38 cm^2) were weighed individually, and the average weights were calculated using digital balance (Secura125-1CN Analytical Balance, China). The thickness of these patches was measured at six different points using thickness gauze (Mitutoyo, Japan). The thickness was measured at three different spots of the films. For each formulation, three randomly selected patches were used [7,8].

Table 1. Formulation design of atenolol buccal patches.

FC	FSM (mg)	HPMC (mg)	SA (mg)	DMSO (%w/w)
F1	125	125	-	
F2	125	125	-	1
F3	125	125	-	5
F4	125	-	125	
F5	125	-	125	1
F6	125	-	125	5

FC – Formulation code, FSM - Fenugreek seed mucilage, HPMC - Hydroxy Propyl Methyl Cellulose and SA – Sodium alginate. Propylene glycol (10 % w/w), drug (25 mg/cm^2) and distilled water (25 ml) were used in all formulations.

Determination of folding endurance:

The folding endurance was determined manually by repeatedly folding the patch at the same place till it broke. The number of times the patches folded at the same place without breaking or cracking gave the value of folding endurance .The experiments were performed in triplicate [9].

Determination of drug content:

The drug contents in each buccal patch was determined by dissolving 1 cm^2 of patches in 100 ml phosphate buffer saline (pH 6.8) and shaken vigorously for 24 h at room temperature. These solutions were filtered through Whatman® filter paper (No. 42). After proper dilution, optical density was measured spectrophotometrically using a UV-VIS spectrophotometer (UV-1700 Double beam spectrophotometer, SHIMADZU Corporation, Japan) at 274 nm against a blank [10-12].

The drug content was estimated from the calibration curve, which was constructed between 1 and 5 µg/ml concentration ranges. The method was validated for linearity, accuracy, and precision. The regression equation for the calibration curve was Y = 0.048 X + 0.002, R^2 = 0.9990.

Measurement of Surface pH:

The surface pH of the patches were determined by placing three patches of each formulation and allowed to swell for 2 h on the surface of an agar plate. The surface pH was measured by using a pH paper placed on the surface of the swollen patch [13]. A mean of three readings was recorded.

Table 2. Physico chemical parameters of prepared atenolol buccal pathes.

FC	Thickness (mm) (X±S.D.)	WV (g) (X±S.D.)	FE	DC (%) (X±S.D.)
F1	0.62±0.03	1.72±0.03	81	99.41±0.13
F2	0.60 0.04	1.74±0.05	86	99.16±0.09
F3	0.58±0.06	1.71±0.04	89	99.33±0.08
F4	0.64±0.05	1.79±0.03	76	98.94±0.12
F5	0.66±0.03	1.80±0.06	83	99.09±0.12
F6	0.65±0.03	1.76±0.04	79	99.18±0.08

WV- Weight variation, FE – Folding Endurance and DC – Drug content. All values are expressed as mean ± standard deviation (n = 3).

Determination of moisture content (MC):

The buccal patches were weighed accurately and kept in desiccator containing anhydrous calcium chloride. After 3 days, the patches were taken out and weighed. The moisture content (%) was determined by calculating moisture loss (%) using the formula [14];

MC (%) = [(Wi - Wf)/ Wi] × 100 …. (1)

Where, Wi and Wf are initial and final weight in mg.

Determination of percentage moisture absorption (MA):

Three buccal patches of 1 cm^2 were weighed accurately and placed in desiccators containing saturated solution of aluminium chloride, keeping 76 % relative humidity inside the desiccator. After three days the patches were removed from desiccators, weighed and percentage moisture absorption was calculated using following formula [14];

MA (%) = [(Wf - Wi)/ Wi] × 100 …. (2)

Where, Wi and Wf are initial and final weight in mg.

In vitro release study:

The *in vitro* release of atenolol from buccal patches was performed using Franz diffusion cell of 1.74 cm^2 diffusion area. The receptor compartment (40 ml) was filled with phosphate buffer saline, pH 6.8, and its temperature was maintained at 37±0.5 °C. The patch was on the cellophane membrane fitted between the donor and receptor compartments of the diffusion cell. A 50 rpm stirring speed was applied using a magnetic stirrer. About 5 ml of the sample from receptor medium was withdrawn at regular intervals and replaced immediately with an equal volume of phosphate buffer saline, pH 6.8. The amount of atenolol released into the receptor medium was determined using UV-VIS spectrophotometer at 274 nm against a blank [14,15].

Preparation of goat buccal mucosa:

The goat cheek pouch was obtained within 2 h of its death from the slaughter house and immediately transported to the laboratory in phosphate buffer saline solution, pH 6.8. The buccal mucosa was excised from goat cheek pouch separated from the full thickness of the tissue after immersion in distilled water and then in phosphate buffer saline, pH 6.8, at 37±1 °C for 2 min. Finally, the mucosa was washed with phosphate buffer saline, pH 6.8 [16,17].

Table 3. Moisture content and Moisture uptake data of different atenolol buccal patches.

FC	pH (X±S.D.)	MC (%) (X±S.D.)	MU (%) (X±S.D.)
F1	6.36±0.03	1.51±0.03	5.83±0.06
F2	6.48±0.01	1.43±0.01	5.76±0.05
F3	6.51±0.02	1.36±0.04	5.89±0.01
F4	6.39±0.02	1.46±0.06	5.96±0.06
F5	6.13±0.03	1.42±0.01	6.03±0.03
F6	6.46±0.03	1.58±0.02	5.88±0.04

FC – Formulation code, MC and MU – Moisture content and uptake (76 % RH). All values are expressed as mean ± standard deviation (n = 3).

Ex vivo permeability study:

The *ex vivo* permeability study were carried out using Franz diffusion cell having diffusion area 1.74 cm^2. The receptor compartment (40 ml) was filled with phosphate buffer saline, pH 6.8, and temperature was maintained at 37±0.5 °C. The goat buccal mucosa was placed between the donor and receptor compartment of the diffusion cell. Over which the buccal patch was placed. A 50 rpm stirring speed was applied using a magnetic stirrer to simulate buccal cavity environment. Five milliliters of the sample from receptor compartment was withdrawn at regular intervals and replaced immediately with an equal volume of phosphate buffer saline, pH 6.8. The amount of drug (atenolol) released into the receptor medium was determined using UV–VIS spectrophotometer at 274 nm against a blank [16,17].

Stability Studies:

The buccal patch formulation having best drug release profile both *in vitro* and *ex vivo*, formulation F3 (atenolol-25 mg/cm^2, fenugreek seed mucilage -125 mg, HPMC-125 mg, propylene glycol-10% w/w, DMSO – 5 % w/w) was stored in borosilicate glass bottles, flushed with nitrogen, and kept in stability chamber at 40 °C/ 75

% RH for a period of six months. A known amount of sample from the formulations subjected to stability testing was analyzed at pre determined time intervals for the drug content, *in vitro* release and *ex vivo* permeation through the goat buccal mucosa [18].

Surface morphology:

The SEM photographs of the (Fig 3) buccal patches containing atenolol indicates a nearly smooth surface and good lamination of the mucoadhesive polymers like Fenugreek seed mucilage, HPMC on the ethyl cellulose backing layer. It shows uniform dispersion of polymeric solution with the drug molecule and confirms perfect binding between the drug containing mucoadhesive layer and the adhesive layer of backing membrane [19].

Statistical analysis:

All evaluations parameters were studied in triplicate. For confirmation of data to be statistical significant, the results were verified with different statistical methods like mean and standard deviations were caluclated [20].

Table 4. *In vitro* drug release profile data of various atenolol buccal patche formulations.

Time (h)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)
0.5	6.06	5.13	4.9	4.9	5.8	5.7
1	13.8	12.7	13.3	12.2	13.9	14.9
2	17.4	18.0	20.0	17.8	18.5	20.9
3	23.6	24.3	25.3	23.2	22.7	28.7
4	30.2	32.7	30.9	28.5	28.0	34.9
5	35.6	38.2	36.5	34.9	34.6	38.3
6	42.5	42.0	41.2	40.1	39.8	45.4
8	48.6	50.9	49.7	46.5	44.3	52.5
12	54.4	61.0	66.2	53.1	58.4	62.5
24	73.5	77.6	81.3	68.4	74.3	80.3

RESULTS AND DISCUSSION:

The solvent casting was found to be successful method for efficient preparation of Atenolol buccal patches as per the formulation design given in Table 1. The thickness and weight variation data are given in Table 2. The thickness of the patches was observed to be in the range of 0.58 ± 0.06 (F3) to 0.66 ± 0.03 mm (F5). The weight of the patches was varied between 1.71±0.04 (F3) to 1.80±0.06 g (F5). These data revealed that almost all atenolol buccal patches showed uniform thickness and weight. This might be preliminarily confirming that all buccal patches possessed uniform dose of the drug. The folding endurance was measured manually. The folding endurance was found to be highest with formulation F3 (89) and lowest for formulation F4 (76) as given in Table 2. The folding endurance of the prepared patches indicates optimum value and therefore good physical and mechanical properties. Drug content of buccal patches was observed to be uniform in the range of 98.94 ± 0.12 (F4) to 99.41 ± 0.13 % (F1) as shown in Table 2. Attempt was made to keep the surface pH close to buccal pH to avoid any irritation in the buccal cavity by proper selection of polymers. The surface pH of the buccal patches was found in the range of 6.13 ± 0.03 (F5) to 6.51 ± 0.02 (F3) as presented tin Table 3.

The percentage moisture content of all the atenolol buccal patches were found to be within the range 1.36 ± 0.04 (F3) to 1.58 ± 0.02 % (F6) as given in Table 3. The low moisture content of the patches protects them well from microbial contamination and also provides stability from brittleness. The moisture uptake test was carried out to check the physical stability of the prepared buccal patches at high humid conditions. The moisture uptake study results found in the range of 5.76 ± 0.05 (F2) to 6.03 ± 0.03 % (F5) as given in Table 3.

Table 5. *Ex vivo* permeation study of different atenolol buccal patch formulations.

Time (h)	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	F6 (%)
0.5	3.2	4.5	4.3	3.1	3.8	4.4
1	9.9	10.8	11.9	8.7	9.9	11.4
2	14.8	15.9	18.3	14.1	13.7	17.2
3	18.6	21.1	22.7	18.2	17.8	23.5
4	22.9	25.2	27.9	21.9	22.2	29.3
5	30.3	31.3	33.6	25.9	25.8	34.5
6	39.2	40.3	38.3	30.3	31.5	41.5
8	44.4	46.2	46.4	36.9	38.2	48.2
12	51.2	51.5	57.3	44.5	49.2	53.8
24	68.5	70.9	73.2	61.9	65.3	70.8

The *in vitro* release data and profile of atenolol from various buccal patches was shown in Table 4 and Fig 1. Highest *in vitro* drug release was observed in formulation F3 (81.3 %) and lowest drug release with formulation F4 (68.4 %). Among six formulations, the *in vitro* release pattern is in the order of F3> F6> F2> F5> F1> F4.The results of *ex vivo* permeation study of atenolol from the buccal patches is shown in Table 5 and Fig 2. It was observed that formulation F3 shows highest drug permeation of 73.2567 % and formulation F4

shows lowest drug permeation of 61.99 % for 24 h. Among six formulations, the drug permeation pattern is in the order of F3> F2> F6> F1> F5> F4. The drug release from the prepared films varied with respect to the polymer composition of films.

Table 6. Stability study data of atenolol buccal patch formulation (F3).

Time	DC (%) (X±S.D.)	DR (%) (X±S.D.)	DP (%) (X±S.D.)
0	99.33±0.08	81.3±4.3	73.2±5.3
1	98.86±0.08	80.0±5.6	71.2±5.6
3	98.54±0.10	78.6±4.4	69.6±4.9
6	98.09±0.06	76.1±5.2	67.1±4.4

Time in month. DC, DR and DP are drug content, release and permeation. All values are expressed as mean ± standard deviation (n = 3).

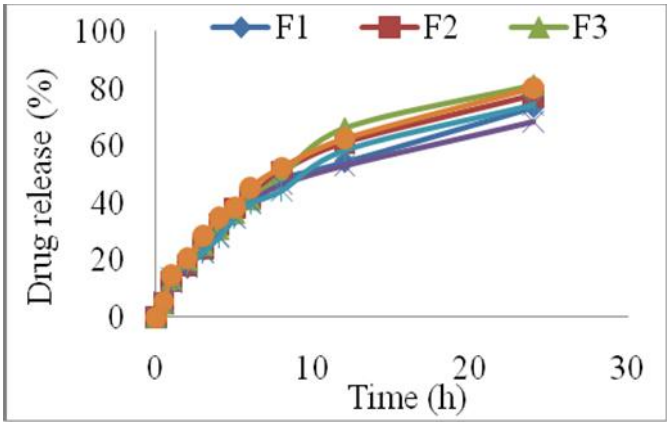

Fig 1. *In vitro* drug release profile of various atenolol buccal patch formulations.

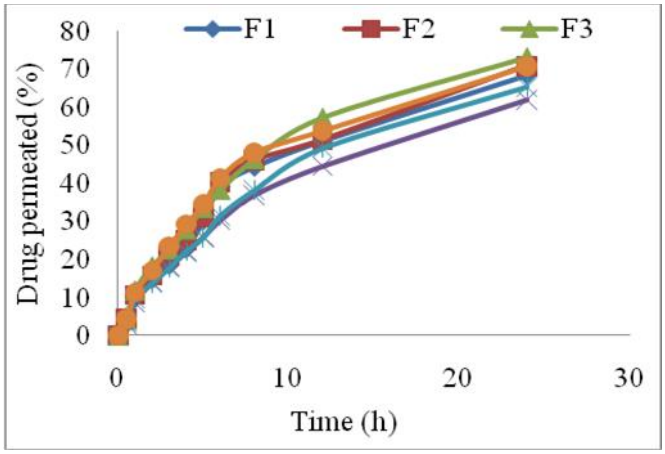

Fig 2. *Ex vivo* drug permeation profile of various atenolol buccal patch formulations.

The stability study for atenolol containing buccal patch formulation F3 (atenolol-25 mg/cm^2, fenugreek seed mucilage-125 mg, HPMC-125 mg, propylene glycol-15% w/w, DMSO- 5%w/w) was conducted as per ICH guidelines and the results were plotted in Table 6. Drug content, *in vitro* drug release and *ex vivo* permeation through goat buccal mucosa results indicates that after six months of stability studies there was no significant difference in drug content, *in vitro* drug release and *ex vivo* permeation through goat buccal mucosa. The surface morphology study of optimized atenolol buccal patches (F3) by SEM revealed that the drug atenolol being uniformely dispersed in the polymeric matrix of buccal pathes, as evident from Fig 3.

CONCLUSION:

The above experimental results of various atenolol buccal patch formulations revealed that the atenolol bucal patch formulation, F3 (atenolol-25 mg/cm^2, fenugreek seed mucilage-125 mg, HPMC-125 mg, propylene glycol-15% w/w, DMSO- 5%w/w) was found to be best optimized formulation as it possessed good physicochemical properties, excellent drug content and best drug release and permeation profile. The optimized formulation was being confirmed as it was found to be stable in various storage conditions as per ICH guidelines. The investigation indicated a new buccal patch formulation for controlled release of atenolol formulated using mucoadhesive polymers. Thus it could be concluded that the new buccal patches of atenolol can be effectively used in safe management of hypertension.

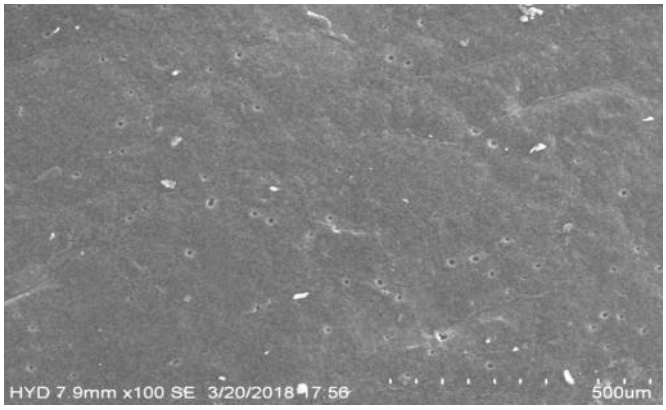

Fig 3. Photograph of optimized atenolol bucal patch (F3) by SEM study.

ACKNOWLEDGEMENTS:

The authors are greatful to Dr. Sivasankar Mohanty for technical help and linguistic support. Authors are also thankful to Authoriy of Seemanta Institute of Pharmaceutical Sciences, Jharpokharia for providing necessary facilities through out the study.

REFERENCES:

1. Malakar J, Sen SO, Nayak AK, Sen KK. Formulation, optimization and evaluation of

transferosomal gel for transdermal insulin delivery. Saudi Pharm J, 2012; 20: 355-363.
2. Das B, Nayak AK, Nanda U. Topical gels of lidocaine HCl using cashew gum and Carbopol 940: Preparation and *in vitro* skin permeation. Int J Biol Macromol, 2013; 62: 514-517.
3. Dreslinski GR, Messerli FH, Dunn FG, *et al*. Haemodynamics, biochemical and reflexive changes produced by atenolol in hypertension. Circulation, 1982; 65:1365-1370.
4. Westfall TC, Westfall DP. Adrenergic agonists and anta agonists: Good man and Gilman's. In: Laurence LB, Bruce AC, Bjorn CK, editors. Goodman & Gilman's: The Pharmacological Basis of Therapeutics. 11th ed. New York: McGraw-Hill; 2014. pp. 2072-2284.
5. Gurpreet SW, Shibba TC, Kaza K. Atenolol Drug Profile. J Assoc Physician India, 2009; 57: 13-16.
6. Attama A, Akpa PA, Onugwu LE, Igwilo G. Novel buccoadhesive delivery system of hydrochlorothiazide formulated with ethyl cellulose hydroxypropyl methylcellulose interpolymer complex. Sci Res Essay, 2008; 3(6): 26-33.
7. Gupta A, Garg S, Khar RK. Measurement of bioadhesion strength of muco-adhesive buccal tablet: design of an *in vitro* assembly. Indian Drugs, 1992; 30: 152-155.
8. Malakar J, Sen SO, Nayak AK, Sen KK. Development and evaluation of microemulsions for transdermal delivery of insulin. ISRN Pharmaceutics, 2011; 1: 1-7.
9. Malakar J, Sen SO, Nayak AK, Sen KK. Formulation, optimization and evaluation of transferosomal gel for transdermal insulin delivery. Saudi Pharm J, 2012; 20: 355-363.
10. Nafee NA, Ahemed F, Borale A. Preparation and evaluation of mucoadhesive patches for delivery of cetylpyridinium chloride (CPC). Acta Pharma, 2003; 199-212.
11. Nayak AK, Pal D, Pradhan J, Ghorai T. The potential of *Trigonella foenum-graecum* L. seed mucilage as suspending agent. Indian J Pharm Edu Res, 2012; 46, 312-317.
12. Nayak AK, Pal D, Pradhan J, Hasnain MS. Fenugreek seed mucilage-alginate mucoadhesive beads of metformin HCl: Design, optimization and evaluation. Int J Biol Macromol, 2013; 54: 144-154.
13. Nayak AK, Pal D, Santra K. Screening of polysaccharides from tamarind, fenugreek and jackfruit seeds as pharmaceutical excipients. Int J Biol Macromol, 2015; 79: 756-760.
14. Patel VM, Prajapati BG, Patel MM. Design and characterization of chitosan-containing mucoadhesive buccal patches of propranolol hydrochloride. Acta Pharm, 2007; 57: 61-72.
15. Adhikari SNR, Nayak BS, Nayak AK, Mohanty B, Formulation and evaluation of buccal patches for delivery of atenolol. AAPS PharmSciTech, 2010; 11(3): 1038-1044.
16. Verma N, Wahi AK, Verma A, Chattopadhayay P. Evaluation of a mucoadhesive buccal patch for delivery of atenolol: *in vitro* screening of bioadhesion. J Pure Appl Microbiol, 2007; 1: 115-118.
17. Adhikari SNR, Satyabrata Panda. Buccal patches of atenolol formulated using fenugreek (*Trigonella foenum-graecum L.)* seed mucilage. Polim Med, *2017; 47*(*1*): 5-11.
18. Sanjay B, Dinesh S, Neha S. Stability Testing of Pharmaceutical Products. J Appl Pharm Sci, 2012; 2(3): 129-138.
19. Sharmila JV, Meera H. Gohil. Scanning electron microscopy study to analyze the morphological characteristics of root surfaces after application of Carisolv gel in association with scaling and root planing: *In vitro* study. J Indian Soc Periodontol, 2012; 16(3): 329-332.
20. Bolton S. Analysis of variance. In: Pharmaceutical Statistics Practical and Clinical Application. New York: Marcel Dekker Inc; 1997. pp. 133- 139.

Conflict of Interest: None
Source of Funding: Nil
Paper Citation: Adhikari SNR, Panda S. A. Atenolol Buccal Patches: *In vitro – Ex vivo* studies. J Pharm Adv Res, 2018; 1(6): 317-322.