Formulations and *In Vivo* Evaluation Studies of Buccal Adhesive Ranolazine Tablets Using Natural Edible Mucoadhesives

DR. TEELAVATH MANGILAL 1*, DR. K.S.K RAO PATNAIK 1

****1 Post Doctoral Fellow, Department of Pharmacy, UCT, Osmania University, Telangana, Hyderabad, 500007,TS, India.***

1.Professor,Department of Chemical Engineering, School of Mechanical, Chemical & Material Engineering, Adama Science and Technology University, Kebele 14, Adama, Ethiopia.

ABSTRACT: The amount of Ranolazine that permeated through the buccal mucosa at defined intervals in a period of four hours was estimated spectrophotometrically. The permeation was similar to the in vitro dissolution studies in most cases and the amount permeated is slightly less than the actual amount of drug dissolved at the similar conditions. Pharmacokinetic parameters of Ranolazine were studied for optimized NBAT formulations, i.v. bolus injection and orally administered core tablets of same batch of NBATs on anaesthetized male New Zealand albino rabbits. Plasma concentration profiles in anesthetized rabbits after the administration of Ranolazine through intravenous, oral and Novel Buccal Adhesive Tablets. Plasma concentration of Ranolazine declined to less than the minimum effective concentration in about 2.5 hours after intravenous administration. Conversely, in oral tablets and NBATs at the same dose MEC was reached after 0.5-1.0 and 1.0-2.0 respectively and remained above the desired level till 2-2.5 and 4-5 hours respectively. Time to reach maximum concentration (T max) for NBAT was 3 - 4 hours whereas it was 1 - 1.5 hour on oral administration. Maximum plasma concentration (C max) for oral (46.9 - 58.9) was found to be less than the NBATs (57.1 - 73.6). The AUC values for after iv administration was 437.53 ± 24.36 (hr)*(ng/ml). On oral administration, the F (bioavailability) values were found to be 0.384±0.36*, 0.367± 0.6**, 0.411±0.1* and 0.353±0.06*respectively for NBAT 3, NBAT 7, NBAT 11 and NBAT 15. Same formulations on buccal administration yielded F values of 0.794±0.09*, 0.766±0.09**, 0.839±0.09**, and 0.744± 0.08** respectively.

Keywords: Mucilages of plant, Ranolazine, Mucoadhesive polymers, sodium alginate and guar gum, First order, Higuchi diffusion or Korsmeyer – Peppas, Male New Zealand albino rabbits.

I.INTRODUCTION

The pharmaceutical companies are presently seeking innovative dosage forms by way of novel drug delivery systems as they represent strategic tool for expanding markets and indications, extending product life cycles and generating newer opportunities[1]. It is a reality and this is illustrated by the fact that around 13% of the current global pharmaceutical market is accounted for NDDS. Among the NDDS, transmucosal drug delivery market recorded second highest growth in the last five years with 171% where as overall market growth stands at 106% [2-3]. The main impediment for oral delivery of these drugs is their inadequate oral absorption due to extensive presystemic metabolism and instability in acidic environment[4-5]. As a result, the full therapeutic potential of many drugs cannot be realized; hence administration through highly expensive and less patient friendly parenteral route is inevitable[6].

In comparison, transmucosal delivery systems exhibit a faster delivery than do transdermal delivery systems. Also, delivery occurs in a tissue that is more permeable than skin and is less variable between patients, resulting in minimal inter subject variability[7]. The absorptive mucosae include buccal, sublingual, palatal, gingival, nasal, pulmonary, rectal, vaginal and ocular routes. On the other hand, in case of nasal delivery, availability of very small surface area for absorption as well as the large variability in mucus secretion could significantly affect drug absorption. Further, severe sensitivity to drugs causes significant irreversible damage to the mucosa. In pulmonary delivery, despite the enormous surface area available for absorption, the major challenge is the reproducible placement of drug in the alveolar region due to the mucociliary clearance, hence not suitable for sustained delivery[8-9]. Among all transmucosal sites, buccal cavity was found to be the convenient and easily accessible site for the local or systemic delivery of drugs. Because of its expanse of relatively immobile smooth muscle, abundant vascularization, direct access to the systemic circulation through the internal jugular vein that bypasses hepatic first pass metabolism, makes it highly promising for delivery of drugs exhibiting poor oral bioavailabilities. Facile removal of formulation, better patient acceptance and compliance are some other

prominent meritorious advantages of buccal adhesive systems. In order to improve bioavailability of administered drug across the buccal mucosa, several bioadhesive tablet systems have been the subject of a growing interest[10-11].

II.MATERIAL AND METHODS

Ranolazine procured from Sun Pharmaceutical Industries Ltd, India as a gift sample, Diazepam from M/S East India Pharmaceutical Works Ltd, Kolkata, India as a gift sample, Gummy exudates of Acacia Arabica Willd from purchased from Local Market, Sodium alginate from Loba chemie, India, Guar gum from E-Merck (India) Hydroxypropyl methyl cellulose (HPMC5cps) and Carbopol 934p from s.d. fine-chem limited, India, Acetone, isopropanol, methonal, chloroform and Buffered formalin from Merck India.

2.1. Preparation of core tablets: Core tablets were formulated by direct compression method by mixing Ranolazine, microcrystalline cellulose, respective mucoadhesive substance, and purified talc. 10 mg of the mixture was weighed and directly compressed using 2.8 mm flat faced punches at the compression force to get tablets with the thickness of 0.8 mm. For human acceptability studies, placebo core tablets were prepared by replacing Ranolazine with the lactose[12-13].

2.2. Preparation of NBATs: Finally, NBATs were prepared by inserting core tablets into the respective cups manually and compressed with little force using 4.5 mm flat faced punches[14-15].

2.3. **Pharmacokinetic studies: *In vivo* animal studies[16-17].**

In vivo studies were conducted on anaesthetized male New Zealand albino rabbits weighing between 1.5 and 1.8 kg. The animals were housed individually in metabolic cages maintained at 25 ± 2 ^{0}C for a period of more than ten days prior to the experiment and provided with standard diet and water. Sixteen rabbits were kept in fasting condition for 24 hours before the start of study but allowed to have free access to water. The approval of the Institutional Animal Ethics Committee was obtained before starting the study and was conducted in accordance with standard institutional guidelines. [Protocol was approved by the Institutional Animal Ethics Committee (GCOP/IAEC/02)] The rabbits were grouped into four, each containing four animals. Group I was used for control, Group II for medicated NBAT, Group III for iv bolus injection and Group IV for core tablets of similar formulation administered orally. Prior to administration, each rabbit was lightly anaesthetized by administering intramuscularly 5-10 mg/kg of xylazine followed in 10 minutes by 35-50 mg/kg of ketamine. The NBATs were administered by pressing manually to either of the buccal mucosa for 30 seconds by exposing core tablet to the mucosa. Intavenous bolus injection (2mg/kg) was injected through marginal ear vein. Core tablets of same batch of NBATs were administered orally. Following induction of anesthesia, a catheter was inserted into the central ear artery of rabbits for blood sample collection. About 2 ml blood sample was collected each time in Eppendorf tubes containing heparin sodium (100 U/ml), 5 min before administration and then at 30, 60, 90, 120, 180, 210, 240, 270, 300, 330, 360 and 390 min after administration. Soon after collection of ea ch blood sample, the cannula was flushed with 0.2 ml of a 10% (v:v) heparin : normal saline solution to prevent blood clotting in the cannula.

Each rabbit was administered with one-third of the initial dose of xylazine and ketamine after every 20 minutes intramuscularly to maintain a light plane of anesthesia. The blood samples were centrifuged at 3000 rpm for 10 minutes immediately after collection to separate the plasma and the retrieved plasma was stored at -20°C until the time of analysis. HPLC analysis Reversed-phase high-performance liquid chromatography was used to quantitate Ranolazine in plasma samples.

2.4. DETERMINATION OF Λ MAX OF RANOLAZINE IN HPLC MOBILE PHASE[18]

Accurately weighed 108.8 mg of Ranolazine equivalent to 100mg of Ranolazine was dissolved in a 100ml volumetric flask and volume was made upto mark with mobile phase. 1ml of this solution was further diluted to 100ml to get the resulting strength of 0.001% w/v was scanned on a JASCO UV-1575 Intelligent UV/VIS Detector.

2.5. Calibration curve of Ranolazine in HPLC Mobile Phase

Prior to the HPLC analysis, a calibration curve was prepared for Ranolazine using diazepam as internal standard. Accurately weighed 54.4 mg of Ranolazine equivalent to 50mg of Ranolazine was dissolved in mobile phase in a 50ml volumetric flask and volume was made up to the mark. 1ml of this solution was further diluted to 100ml with mobile phase. This was the stock solution having concentrations 10 µg/ml. Calculated quantities of aliquots of the solution were diluted to individually with the mobile phases to give concentrations of 4 to 400ng/ml Ranolazine. An aliquot of 1.0 ml of the solution was mixed with 200µl of 1.0M of Na_2Co_3 followed by 5ml of mixture of organic solution of hexane, chloroform and isopropanol (60:40:5, v/v/v) containing 15ng/ml of diazepam (internal standard) in a chemically cleaned screw capped glass tube. It was vortexed for 2 minutes and then centrifuged for 10 minutes at 4000 rpm. A 4.0 ml of organic layer was separated and evaporated with air at 60^{O}C until dried. The residue was then dissolved by vortexing for 30 seconds with 120µl of mobile

phase from which 30µl sample was injected through Microliter # 702 injector, Hamilton (Switzerland) into BDS Hypersil C18 (4.6 mm x 150 mm) (Thermo Electron corporation) column driven through JASCO PU 1580 HPLC pump. The mobile phase used was methanol:water (80:20) mixture containing 2.8 mM triethylamine (filtered through a 0.45µM membrane filter). The flow rate was fixed at 1.2ml/min and detection was measured at 239 nm using JASCO UV-1575 detector. The chromatographic peaks was automatically integrated and recorded by Chromatographic stations for Windows 1.7 data module (Data Apex; Prague, Czech Republic). This method enabled the baseline separation of the drugs free from interferences with isocratic elution and was linear in the clinical range 4-400 ng/ml. Under the operated conditions, Ranolazine and diazepam had retention times of approximately 5.267 and 3.813 minutes, respectively. Results showed linearity related to concentration at the range of 5 ng to 400 ng. The linear equation for the concentration vs the ratio of peak area was y = 0.0078*x*-0.0003 with correlation coefficient of 0.9994 (n=4).

2.6. Quantitative analysis of Ranolazine in plasma

An aliquot of 1.0 ml of plasma was mixed with 200µl of 1.0M of Na_2Co_3 followed by 5ml of mixture of organic solution of hexane, chloroform and isopropanol (60:40:5, v/v/v) containing 15ng/ml of diazepam (internal standard) in a chemically cleaned screw capped glass tube. It was vortexed for 2 minutes and then centrifuged for 10 minutes at 4000 rpm. A 4.0 ml of organic layer was separated and evaporated with air at 60^oC until dried. The residue was then dissolved by vortexing for 30 seconds with 120µl of mobile phase from which 30µl sample was injected through Microliter # 702 injector, Hamilton (Switzerland) into BDS Hypersil C18 (4.6 mm x 150 mm) (Thermo Electron corporation) column driven through JASCO PU 1580 HPLC pump. The mobile phase used was methanol:water (80:20) mixture containing 2.8 mM triethylamine (filtered through a 0.45µM membrane filter). The flow rate was fixed at 1.2ml/min and detection was measured at 239 nm using JASCO UV-1575 detector. The chromatographic peaks was automatically integrated and recorded by Chromatographic stations for Windows 1.7 data module (Data Apex; Prague, Czech Republic). This method enabled the baseline separation of the drugs free from interferences with isocratic elution and was linear in the clinical range 0-400 ng/ml.

2.7. Pharmacokinetic studies [19]

Pharmacokinetic parameters were evaluated by using ThermoKinetica 4.4, PK/PD analysis, Thermoelectron Corporation. Intravenous data was studied using one compartment model where as oral and buccal data was studied by Loo-Riegelman 2 compartmental method. Parameters such as C_{max}, T_{max}, AUC_{0-t}, AUC total, $AUMC_{0-t}$, AUMC total, *K*el, *t*1/2, MRT, CL, Vd, K12, K21 etc were calculated. Oral and buccal bioavailabilities were also calculated after normalizing the dose with the intravenous dose. The plasma concentration profiles were represented graphically in Figs 62 – 65. The means of all data were presented in Table 19 with their standard error (mean ± S.E.). Parameters were analyzed to determine statistical significance. The mean concentration at each time point was compared for statistical difference using a non-paired Student's *t*-test. In addition, the test was also conducted for T_{max} and logarithmically transformed values for C_{max} and AUC.

2.8. STATISTICAL ANALYSIS [20]

2.8.1.T-test using Graph Pad software

The Student's *t* -test is a test developed by W. S. Gossett who used the pseudonym "Student" to publish this statistical test in 1908. It is used to express confidence intervals for a set of data and to statistically compare the results of different experiments.

The true mean is denoted asµ. From a small number of data points it is not possible to determine either µ or σ. Instead, we have x_{mean} and s. We would like to be able to state the probability that the true value µ is within some quantity of $x_{mean.}$ The confidence interval does this in the form $\mu = x_{mean} \pm t\ s / \sqrt{n}$ and may stated at a certain probability such as 90%, 95%, or 99%, etc.

III.RESULTS AND DISCUSSION

Pharmacokinetic parameters of Ranolazine were studied for optimized NBAT formulations, i.v. bolus injection and orally administered core tablets of same batch of NBATs on anaesthetized male New Zealand albino rabbits and were reported. During the period of experiment the NBATs remained at the site of application. Prior to the HPLC analysis, a calibration curve was prepared for Ranolazine using Diazepam as internal standard. Under the operated conditions, Ranolazine and Diazepam had retention times of approximately 5.267 and 3.813 minutes, respectively. Results showed linearity related to concentration at the range of 5ng to 400ng as

represented in Fig.1 and Table1.

Table (1): calibration Curve for Ranolazine in HPLC Mobile Phase Pharmacokinetic Parameters

Parameters	Units	iv	Oral (Core 3)	NBAT 3	Oral (Core 7)	NBAT 7	Oral (Core 11)	NBAT 11	Oral (Core 15)	NBAT 15
Dose	ng		3403920	3403920	3395590	3395590	3406250	3406250	3362590	3362590
Volume	ml		7631.21	7668.23	7632.08	7668.23	7668.23	7668.23	7631.18	7631.21
Kel			0.982±0.01	0.985±0.01	0.98±0.03	0.985±0.01	0.98±0.02	0.98±0.03	0.982±0.02	0.982±0.04
K12			0.602±0.02	0.610±0.02	0.60±0.05	0.610±0.03	0.61±0.03	0.61±0.02	0.602±0.02	0.602±0.03
K21			0.467±0.01	0.466±0.01	0.46±0.03	0.466±0.04	0.46±0.01	0.46±0.03	0.467±0.02	0.467±0.02
C_{max}	ng/ml	419.7± 8.26*	53.7±4.29	67.9±7.23*	46.9± 6.7	73.5±4.21	58.9±7.54	72.4±3.46	55.6±6.18	57.1± 5.49**
T_{max}	hr	---	1.5±0.25	3.5±0.25	1± 0.25	3±0.25	1.5±0.25	3.5±0.25	1±0.25	3± 0.25
AUC_{last}	(hr)*(ng/ml)	385.5±23.7*	143.18±16.58	241.9±37.8	134.7± 19.1	250.37±17.6	164.1±31	259.6±29	131.63±16.2*	221.81± 31.5
AUC_{extra}	(hr)*(ng/ml)	51.9± 2.41	36.13±2.19	129.5±6.54	36.74± 5.22	107.76±10.3	27.9±3.29	132.4±6.1*	33.55±6.29	125.80± 17.8
AUC_{tot}	(hr)*(ng/ml)	437.5± 2436*	179.32±13.7	370.98±35	171.5± 2.7**	358.14±42.3	192.1±17.8	392.0±61.9	165.18±34.6	347.6± 43.6**
$AUMC_{last}$	(hr)^2*(ng/ml)	545.1± 46.2	320.3±22.32	754.8±41.3	312.9± 44.6	741.2±39.24	366.4±26.6	810.6±41.6	278.9±16.54	687.9 ± 76.4
$AUMC_{extra}$	(hr)^2*(ng/ml)	570.6± 48.6	442.1±27.43	1934.5±111	431.8± 61.5	1518.3±67.3	323.7±44.6	1898.9±98	408.9±27.65	1642± 156.9
$AUMC_{tot}$	(hr)^2*(ng/ml)	1115.74± 89*	762.4±26.57	2689.3±121	744.7± 105	2259±103.2*	690.1±62.3	2709±171.8	687.8±42.53*	2330± 271.3
$T_{1/2}$	hr	3.10± 0. 03*	3.97±0.348	5.88±0.071	3.63± 0.19**	5.26±0.075	3.52±0.031	5.43±0.061	3.94±0.022	4.54± 0.26**
MRT	hr	2.55± 0.06	4.25±0.071	7.24±0.034	4.34± 0.106	6.31±0.0484	3.59±0.037	6.91±0.084	4.16±0.0265	6.70± 0.162
Clearance	ng*hr/(ng/ml)	7267± 56.4	18935.9±762.3	9152.9±411	19796± 141	9481±151.2	17675±348	8661.13±21	20556±645.7	9768.2± 89.8
Vz	ng/(ng/ml)	32562±103.2	108606±378.9	77710±142	103930±66	71952±716	898186±42	67879±382.9	116905±978	64004.4±581
Vss	ng/(ng/ml)	18533.8±167	80509.2±241.6	66352±333	85953±244	59815±156	63492±316	59859±102.7	85595.8±275	65475.2±100
A		3.8758±0.09*	2.975±0.07	3.480±0.07*	3.178±0.08	3.508±0.06*	2.980±0.04	3.653±0.06	2.904±0.08	3.932±0.04
B	1/hr	-0.2232±0.01	-0.1773±0.01	-0.117±0.02	-0.190±0.01	-0.131±0.01	-0.19±0.02	-0.1276±0.01	-0.1758±0.02	-0.1526±0.01
t50%	hr		0.895±0.07	2.166±0.09	0.817±0.07	1.945±0.05	0.955±0.08	2.137±0.05	0.699±0.02	2.042±0.09
t90%	hr		1.885±0.11	3.471±0.16	2.657±0.09	3.221±0.10	1.942±0.09	3.477±0.08	1.779±0.03	4.146±0.18
F			0.384±0.36*	0.794±0.09*	0.367± 0.6**	0.766±0.09**	0.411±0.1*	0.839±0.09**	0.353±0.06*	0.744± 0.08**

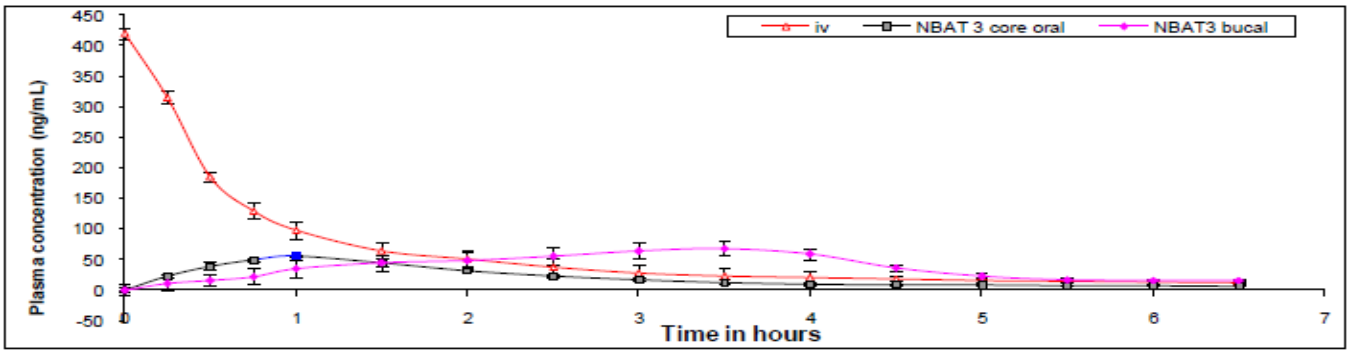

Fig.1.Plasma Concentration Profiles of Ranolazine in Anaesthetized Rabbits (NBAT 3)

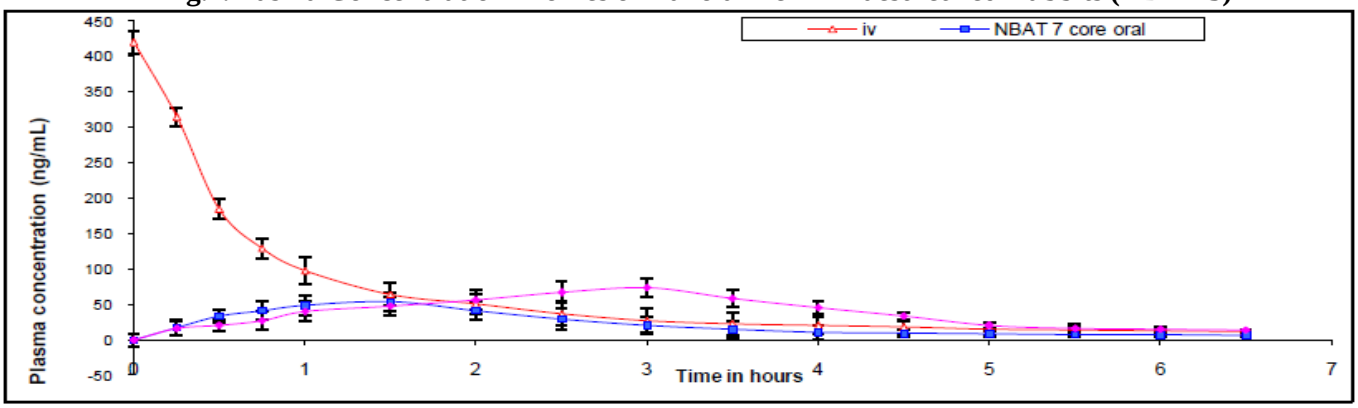

Fig.2. Plasma Concentration Profiles of Ranolazine in Anaesthetized Rabbits (NBAT 7)

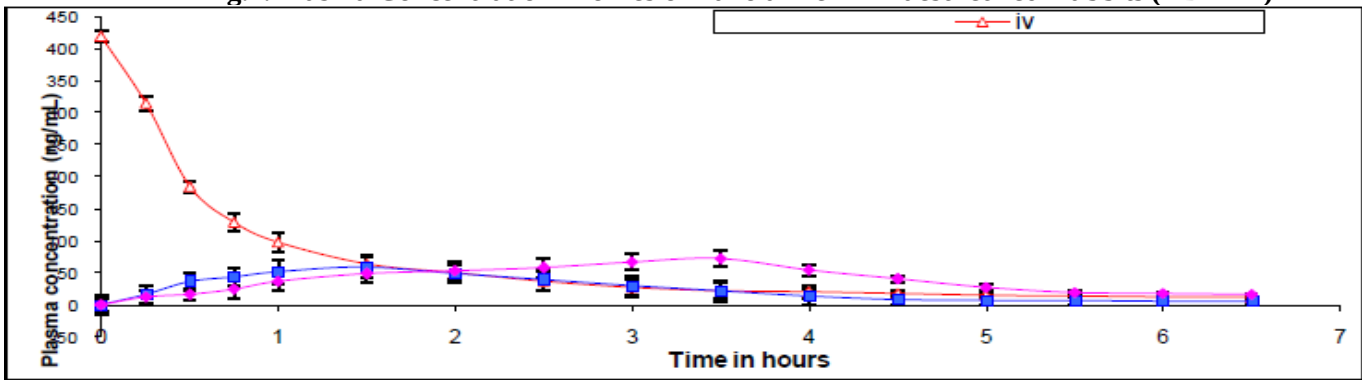

Fig.3. Plasma Concentration Profiles of Ranolazine in Anaesthetized Rabbits (NBAT 11)

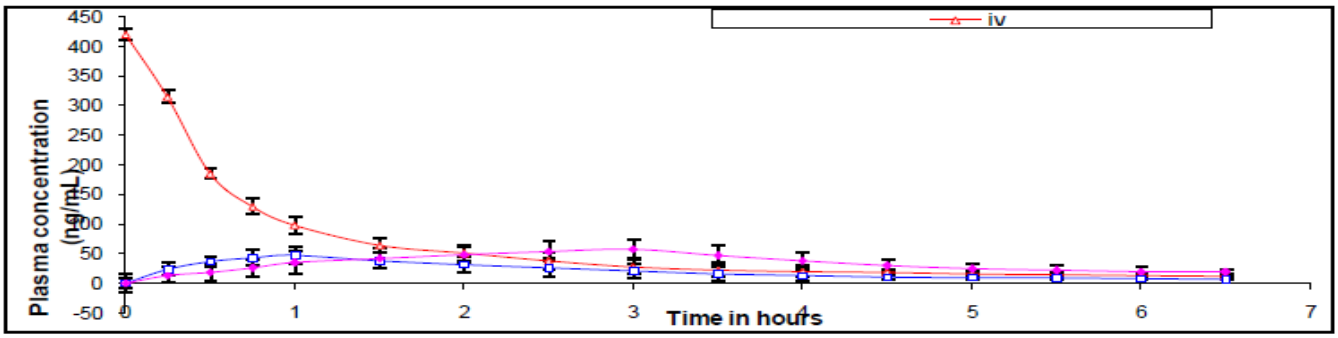

Fig.4. Plasma Concentration Profiles of Ranolazine Anaesthetized Rabbits (NBAT 15)

Figures 1 to 4 represents the plasma concentration profiles in anesthetized rabbits after the administration of Ranolazine through intravenous, oral and Novel Buccal Adhesive Tablets. Plasma concentration of Ranolazine declined to less than the minimum effective concentration in about 2.5 hours after intravenous administration. Conversely, in oral tablets and NBATs at the same dose MEC was reached after 0.5-1.0 and 1.0-2.0 hrs respectively and remained above the desired level till 2-2.5 and 4-5 hours respectively. Time to reach maximum concentration (T_{max}) for NBAT was 3 - 4 hours whereas it was 1 - hour on oral administration. Maximum plasma concentration (C_{max}) for oral (46.9 - 58.9) was found to be less than the NBATs (57.1 - 73.5). The AUC values for after iv administration was 437.53 ± 24.36 (hr)*(ng/ml). On oral administration, the F (bioavailability) values were found to be $0.384\pm0.36^{*}$, $0.367\pm 0.6^{**}$, $0.411\pm0.1^{*}$ and $0.353\pm0.06^{*}$ respectively for NBAT 3, NBAT 7, NBAT 11 and NBAT 15. Same formulations on buccal administration yielded F values of $0.794\pm0.09^{*}$, $0.766\pm0.09^{**}$, $0.839\pm0.09^{**}$, and $0.744\pm 0.08^{**}$ respectively. Statistical analysis of all the parameters suggests that the methods and the dosage forms are reliable and highly reproducible.

CONCLUSION

In vitro release studies showed that the tablet formulations containing natural mucoadhesive agent exhibited sustained release kinetics. Further, the amount of drug that leached through the backing layer was also found to be very minimal. *Ex-vivo* permeation studies through the porcine buccal mucosa also exhibited similar release profile. It was found that the release is delayed as the amount of polymer is increased in the core tablets. *In vivo* studies on the anaesthetized New Zealand albino rabbits showed good absorption profiles with reduced excretion rates.

REFERENCES

1.Y.W. Chien, Concepts and system design for rate-controlled drug delivery, in: Y.W. Chien (Ed.), Novel Drug Delivery Systems, Marcel Decker, Inc; New York, (1992), 1-42.
2.M.J. Rathbone, G. Ponchel, F.A. Ghazali, Systemic oral mucosal drug delivery and delivery systems, in: M.J. Rathbone (Ed.), Oral Mucosal Drug Delivery, Marcel Decker, New York, 1996, 74,241-284.
3.Y. Sudhakar, K. Kuotsu, A.K. Bandyopadhyay, Buccal bioadhesive drug delivery-A promising option for orally less efficient drugs, J. Contr. Rel., (2006) ,114 (1),15-40.
4.J. Hao, P.W.S. Heng, Buccal delivery systems, Drug Dev. Ind. Pharm., 2003, 29(8),821 – 832.
5.P.L. Soo, L. Luo, D. Maysinger, A. Eisenberg, Incorporation and release of hydrophobic probes in biocompatible polycaprolactone-block-poly (ethylene oxide) micelles: Implications for drug delivery, Langmuir., 2002,18 ,9996-10004.
6.P. Perugini, I. Genta, B. Conti, T. Modena, F. Pavanetto, Periodontal delivery of ipriflavone: new chitosan/PLGA film delivery system for a lipophilic drug, Int. J. Pharm., 2003,18,1-9.
7.S. Senel, G. Ikinci, S. Kas, A. Yousefi-Rad, M.F. Sargon, A.A. Hıncal, Chitosan films and hydrogels of chlorhexidine gluconate for oral mucosal delivery, Int. J. Pharm., 2000,193,197-203.
8.D. Tiwari, D. Goldman, R. Sause, P.L. Madan, Evaluation of Polyoxyethylene Homopolymers for Buccal Bioadhesive Drug Delivery Device Formulations, AAPS PharmSci., 1999,1,1-8.
9.R. Anders, H. Merckle, Evaluation of laminated mucoadhesive patches for buccal drug delivery, Int. J. Pharm.,1989 ,49,231-240.
10.R. Anders, H.P. Merckle, W. Schurr, R. Ziegler, Buccal absorption of Protirelin: an effective way to stimulate Thyrotropin and Prolactin, J. Pharm. Sci., 1983,72,1481-1483.
11.J.H. Guo, Investigating the surface properties and bioadhesion of buccal patches, J. Pharm. Pharmacol., 1994 ,46,647-650.
12.K. Danjo, H. Kato, A. Otsuka, K. Ushimaru, Fundamental study on the evaluation of strength of granular particles, Chem. Pharm. Bull., 1994 ,42,2598-2603.
13.M. Ishida, N. Nambu, T. Nagai, Mucosal dosage form of lidocaine for toothache using hydroxypropyl cellulose and carbopol, Chem. Pharm. Bull.,1982 ,30,980-984.
14. N.A. Nafee, F.A. Ismail, A. Nabila, Boraie, L.M. Mortada, Mucoadhesive buccal patches of miconazole nitrate: *in vitro/in vivo* performance and effect of ageing, Int. J. Pharm.,2003,264,1-14.
15.T. Save, U.M. Shah, A.R. Ghamande, P. Venkatachalam, Comparative study of buccoadhesive formulations and sublingual capsules of

nifedipine, J. Pharm. Pharmacol.,1994, 46(3),192-195.
16.A.H. Shojaei, X. Li, Mechanisms of buccal mucoadhesion of novel copolymers of acrylic acid and polyethylene glycol monomethylether monomethacrylate, J. Control. Rel.,1997,47, 151-161.
17.S. Miyazaki, A. Nakayama, M. Oda, M. Takada, D. Attwood, Chitosan and sodium alginate based bioadhesive tablets for intraoral drug delivery, Biol. Pharm. Bull.,1994,17, 745-747.
18.C. Remunan-Lopez, A. Portero, J.L. Vila-Jato, M.J. Alonso, Design and evaluation of chitosan/ethylcellulose mucoadhesive bilayered devices for buccal drug delivery, J. Control. Rel.,1998,55,143-152.
19.B. Taylan, Y. Capan, O. Guven, S. Kes, A.A. Hincal, Design and evaluation of sustained release and buccal adhesive propronolol hydrochloride tablets, J. Control. Rel., 1996, 38(1), 11- 20.20.Cochran,William G;Cox,Gertrude M.,Experimental design.,New York:Wiley.1992,2nd ed.