Section A-Research paper
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In vivo pharmacokinetic study of felodipine microparticles-loaded rectal dosage form

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Abstract

The objective of present study was to develop rectal formulation of based on microparticles and perform in vivo study of developed rectal formulation in rats. The formulated suppositories were examined for different evaluation parameters like weight variation, disintegration time, in vitro dissolution study, stability study and pharmacokinetic study. The disintegration time and percent cumulative drug release of the suppositories were determined in the range between 13.69±0.93 min to 20.94±0.63 min and 88.23±0.91 to 96.47±0.02 respectively. During *In vivo* pharmacokinetic study in male Sprague–Dawley Rats, the relative bioavailability of rectally administered felodipine microparticles loaded suppository was found 148.15%. The study reveals that rectal administration of felodipine as microparticles loaded suppository was an alternate route of administration.

Key words: Felodipine, Microparticle loaded suppository, Fusion method, *In vivo* Pharmacokinetic study

1. Introduction

Rectal route comprise the avoidance of first pass elimination, the possibility of rate controlled drug delivery and absorption enhancement.[1] Suppository presents the common dosage form of rectal administration. Conventional suppositories, which may reach to the end of the canal of the application site, because of its poor mucoadhesive properties, lose drugs at that level, and may also allow the carried

drugs to undergo the first pass effect. To solve these problems associated with the use of conventional solid suppository, it would be desirable to develop a novel solid suppository, which had mucoadhesive properties to such level that it would attach to the site of application, the rectal mucous membrane and do not reach to the end of the colon.[2,3] Residence time or mucoadhesion may be controlled by mixing two or more water-soluble polymers with different mucoadhesion.[4] Ketoprofen chitosan granules and also theophylline and oxyphenbutazone microcapsules containing polyethylene glycol suppositories were reported.[5,6] Rectal availability of cefuroxime sodium mucoadhesive microspheres was studied.[7] Rectal administration can modify the pharmacokinetics of antihypertensive drugs.[8]

Felodipine was selected here as a model drug, since it undergo extensive first-pass degradation. Felodipine is a long-acting 1, 4-dihydropyridine calcium channel blocker. It acts primarily on vascular smooth muscle cells by stabilizing voltage-gated L-type calcium channels in their inactive conformation. Felodipine is used to treat mild to moderate essential hypertension.[9]

The objective of the present study was to comparatively evaluate felodipine microparticles loaded rectal suppository with oral suspension of felodipine and felodipine loaded suppository for pharmacokinetic study in rats.

2. Materials and Methods

2.1. Materials

Tamarind kernel polysaccharide (TKP) was obtained from Hindustan Gums and Chemicals Pvt. Ltd. (Bhiwani, India). Felodipine was obtained as a gift sample from Cipla Ltd. (Mumbai, India) Sodium alginate and calcium chloride were obtained as gratitude samples from Thomas Baker Chemicals Pvt. Ltd (Mumbai, India) and poloxamer 188 (P188) from Mylan Laboratories (Nasik, India). Freshly excised rectal cavity was obtained from the local butcher shop (Nasik, India). Methanol and acetonitrile used was HPLC grade from Rankem Ltd, India. Water used was HPLC grade generated from Milli-Q purification system. All other chemicals were of reagent grade and were used as such.

2.2. Formulation of felodipine alginate TKP microparticles:

Felodipine microparticles were formulated with the use of ionic gelation technique. Calcium chloride was used as cross-linker. Different concentrations of aqueous solutions of sodium alginate (2- 3%w/v) mixed with 1% w/v aqueous gel of TKP and felodipine (1% w/v). The resultant mixture was mixed drop-wise using pipette into the solution of calcium chloride (5-10% w/v) using 26 gauge needle. Curing time was set to 15 min and henceforth mixed drops were retained in calcium chloride solution for same time span to crop rigid microparticles. The microparticles so prepared were harvested by decantation method and further dried overnight at room temperature.[10,11]

Felodipine alginate TKP microparticles were prepared by ionic gelation method using calcium chloride as cross-linking agent. Microparticle batches were prepared with different proportions of core to coat materials (drug: polymer = 1:2:1, 1:2.5:1 and 1:3:1 (w/v)). Felodipine (1% w/v) was dispersed in a mixture containing

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different concentrations of aqueous solutions of Sodium alginate (2-3%) and 1% aqueous gel of TKP. The resulting mixture was added drop-wise into aqueous solution of calcium chloride (5-10%) using 24 gauge needle. The added droplets were retained in $CaCl_2$ solution for 15 min to complete curing reaction and to produce rigid microparticles. The microparticles so prepared were collected by decantation technique and then air-dried overnight at room temperature.[12]

Considering the previous research work,[12] the selected batch has concentrations of alginate 2.559%, TKP 1% and calcium chloride 6.932%. The selected batch of felodipine loaded microparticles was found to have 94.271% EE (predicted 96.071%) and 93.995% mucoadhesion strength (predicted 95.682%).

2.3 In vitro release study of felodipine alginate TKP microparticles

Optimized batch of felodipine microparticles equivalent to 5 mg of felodipine was weighed accurately and subjected to release rate study in 6.8 phosphate buffer using USP dissolution test apparatus II (Model: Tablet Dissolution Test Apparatus, Labindia) with autosampler. Felodipine was used as control and subjected to release rate study by weighing 5 mg of it. Sampling volume was 5 ml for both with sampling interval 15, 30,45,60,90,120,180 and 240 min. The drug concentration in the sample was determined from the standard curve of the drug in ethanol: pH 6.8 phosphate buffer at 360.4 nm.

2.4. Preparation of felodipine alginate TKP microparticles loaded suppository

Optimized formulation of microparticles was further developed as suppository with different concentrations of Poloxamer 188/PG as 80%/20%, 70%/30%, 50%/50% for rectal administration. Suppository contains 5% microparticles and 95% suppository base. Suppositories were developed by fusion method.

Poloxamer 188 and PG were mixed and heated up to 55^{o}C. Optimized batch of felodipine loaded alginate TKP microparticles was then slowly added to the solution with continuous agitation. The resulting solution was then poured into suppository mould and cooled down to 25^{o}C.[13]

2.5 Evaluation of felodipine and Felodipine alginate TKP microparticles loaded suppository

2.5.1 Determination of melting point of suppository bases

Melting point of different ratios of poloxamer bases and/or propylene glycol was determined by obtaining DSC thermogram on a heating speed of 10^{o}C/min.

2.5.2 Weight variation: 20 suppositories were weighed and after determination of weight of 20 suppositories, average weight of suppository was calculated. Weight of each suppository was then determined using electronic balance. Not more than two suppositories should deviate from 5 % of average weight.[14]

2.5.3 ***In vitro*** **disintegration test of suppository**

Three suppositories of each type (with different ratios of poloxamer bases and/or propylene glycol) were placed on the lower perforated discs and then the devices were inserted into cylinder and to the sleeves. This assembly was placed vertically 90 mm below the surface of buffer in a vessel containing slow stirrer, temperature measuring device and 4 liters of pH

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6.8 phosphate buffer at 37ºC. After each 10 min each apparatus was inverted to check the disintegration of suppository. Disintegration of suppository is considered to be complete when suppository is completely dissolved or disintegrated into its components or sink to the bottom or become soft with considerable change in shape.[15]

2.5.4 *In vitro* **release study**

Each suppository containing required amount of microparticles was placed into semi permeable membrane tube. Tube was sealed at both ends by tying with thread to avoid leakage of contents and then subjected to release rate study in 500 ml pH 6.8 phosphate buffer using USP dissolution test apparatus II (Model: Tablet Dissolution Test Apparatus, Labindia) with autosampler. 5mL sample solution was pipette out at set time intervals. Same volume of dissolution medium was added to maintain sink condition. Drug concentration in sample was determined by using spectrophotometer at 360.4 nm developed analytical method for determination of drug.[16]

2.5.5 Stability study

To carry out short term stability study of suppository, the suppositories were individually wrapped in aluminum foil and packed in cardboard boxes and were kept at refrigeration temperature (4°C) for 6 w. Samples are taken after 6 w for physical appearance and drug content estimation.[17]

2.5.6 Pharmacokinetic study

Subjects: Prior to experimental study male Sprague-Dawley Rats having body weights in range of 350-400 g were fasted for 36 hours but with free access to water. The animals were classified into 3 groups with 5 animals in each group. The protocol for this investigation was approved by the Institutional Animal ethics committee in accordance with the disciplinary principles and guidelines of Committee for the purpose of control and supervision of experiments on animals (CPCSEA/IAEC/SPTM/P-43/2015).

The animals received oral suspension through an oral tube. Pure drug suppository, felodipine tamarind alginate microparticles loaded suppository were inserted into the rectum (with equivalent dose of 10 mg of felodipine/kg). After administration of formulations through different routes 0.5 ml of blood sample will be collected through retro- orbital plexus at each time at interims of 0.5, 1, 2, 3, 4, 5, 6, 8, 12 and 24 h. 0.1ml anticoagulant (Heparin, 25,000 I.U in 5 ml) was mixed with each blood sample and processed further for centrifugation for 15 min and at speed of 3000 rpm. Ethyl acetate (0.4 ml) was mixed with plasma and again processed for centrifugation with speed of 10000 rpm and temperature of 4˚C. As process of centrifugation is complete, separation of organic layer takes place. This organic layer was vaporized under nitrogen. To this processed plasma mobile phases were added and analyzed through HPLC. The mobile phase was made up of Methanol and

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0.01M KH_2PO_4 pH 3.5 (75:25) and delivered into HPLC apparatus at speed of 1ml/min and analysed at wavelength 240 nm. Column used was Kromasil C18, 150*4.6mm.[18]

Assessment of pharmacokinetic analysis parameters

Kinetica 5.0® software was used to assess Noncompartment pharmacokinetic analysis parameters such as Cmax, Tmax as well as AUC.

$$\%Frel = \frac{AUCtest}{AUC\ std} \times 100\text{-----(1)}$$

Where % Frel = Relative bioavailability in percent
AUC test = AUC after microparticle loaded suppository administration(test)
AUC std = AUC after drug loaded suppository administration (std)

2.5.7. Stability study

For short term stability study of suppository, the suppositories were individually wrapped in aluminium foil and packed in cardboard boxes and were kept at refrigeration temperature (4°C) for 6 w. Samples are taken after 6 w for physical appearance and drug content estimation.

3. Result and Discussion:

3.1 Determination of melting point of suppository bases

Poloxamer 188 was mixed by heating with propylene glycol to form homogeneous mixture in the ratio of 80%:20%; 70%:30%; 50%:50%. DSC analysis of mixtures (Fig.1) showed that increase in ratio of propylene glycol depresses melting temperature of mixture. Different ratios of Poloxamer 188: Propylene glycol has shown melting point at 42ºC, 35 ºC, 11 ºC respectively.

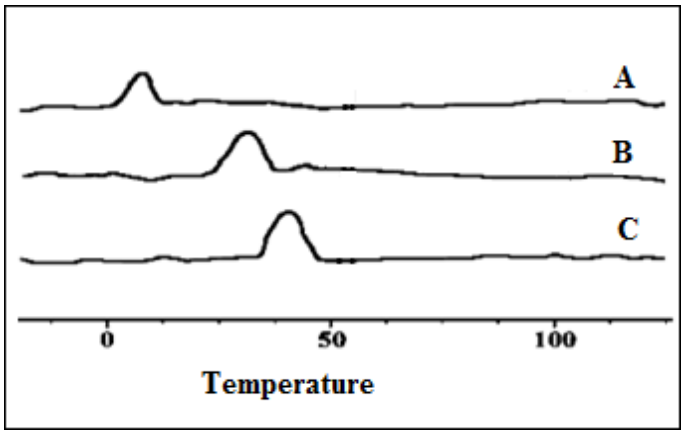

Fig 1: DSC Curves of suppositories Poloxamer 188/Propylene glycol A) 50%/50% B) 70%/30% C) 80%/20%

3.2 Weight variation

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The weight variation study for suppositories was carried out. This parameter was in acceptable range (<5%).

3.3 Disintegration test of drug loaded and microparticles loaded suppository

Table 1: Disintegration time of suppository formulations

Sr. No.	Formulation	Disintegration Time (min)
1	Felodipine suppository (70:30)	15.43±0.32
2	Felodipine alginate TKP microparticles suppository 1 (80:20)	20.94±0.63
3	Felodipine alginate TKP microparticles suppository 2 (70:30)	17.76± 0.43
4	Felodipine alginate TKP microparticles suppository 3 (50:50)	13.69±0.93

3.4 *In vitro* release study

From *in vitro* release study (Fig.2), as compared to felodipine alginate TKP microparticles, microparticles loaded suppositories showed enhanced release of felodipine. Hydrophilic portions of the poloxamers probably contribute to increase the dissolution of felodipine. Due to the amphiphilic structure of poloxamers, these are developing the micellar core surface area accessible to felodipine molecules.[13] It was found that suppository 3 with [P 188/PG (50%/50%)] had highest dissolution rate as compared to other suppositories. However, there were no significant differences among the dissolution rates of all three combinations of suppositories. Hence suppository 2 with P 188/ PG (70%/30%) ratio was selected for *in vivo* release study.

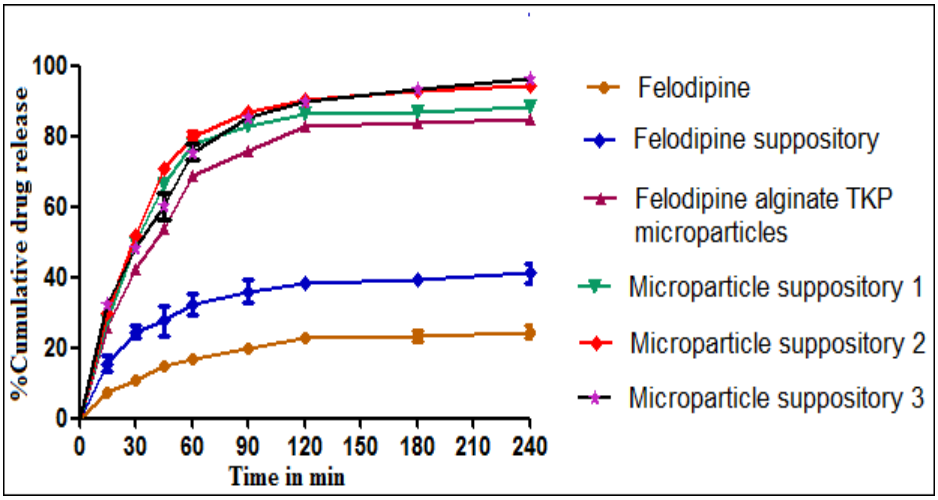

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Fig. 2. Release profiles of felodipine formulations

Table 2: Dissolution Kinetic Parameters

Dissolution Kinetic Parameters			
Formulation	**Release Exponent (n)**	**Kinetic constant (k)**	**Correlation Coefficient (r^2)**
Felodipine loaded microparticles	0.5273	0.9947	0.9425
Felodipine microparticles loaded suppository P188/PG (80/20%)	0.5872	1.1103	0.8103
P188/PG (70/30%)	0.5899	1.1285	0.8290
P188/PG (50/50%)	0.5926	1.1109	0.9058

Higher values of kinetic constant, k represents higher dissolution rate. Penetration of water molecules into water soluble polymeric microparticles results into swelling and erosion of swellable polymer matrix. Mechanism involved in drug release is diffusion, erosion and dissolution controlled and represent non-fickian type drug release (as values of release exponent were $0.43 < n < 0.85$).

3.5 Stability study

Physical characteristics as well as drug content in suppository remained unchanged after storage at freezing temperature for 6 weeks to evaluate stability.

3.6 Pharmacokinetic parameters of felodipine formulations

The pharmacokinetics of felodipine formulation was studied in rats. The results obtained from the pharmacokinetic analysis are represented in Fig 3 demonstrating felodipine serum concentration after microparticle suppository, pure drug suppository and oral suspension administration. Outcomes of in vivo study have shown that rectal formulations achieved greater serum concentrations of felodipine than oral suspension as revealed by Cmax and AUC values. Resultant pharmacokinetic analysis parameters, peak concentration (Cmax, µg/ml), Tmax (hrs) and AUC are stated in following table 3.

Table 3: Pharmacokinetic parameters of felodipine formulations (n=5)

Parameters	Felodipine oral Suspension	Felodipine loaded suppository	Felodipine alginate tamarind microparticlesloaded suppository
Cmax (µg/ml)	1.22±0.16	2.04±0.71	3.24±0.41
Tmax(hours)	2	2	3
AUCtotal (µg.h/ml)	14.28±1.28	24.81±5.43	38.09±2.79

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AUC0-∞ (µg.h/ml)	14.91±3.54	28.39±4.56	42.06±4.67
Frel (%)	-	-	148.15

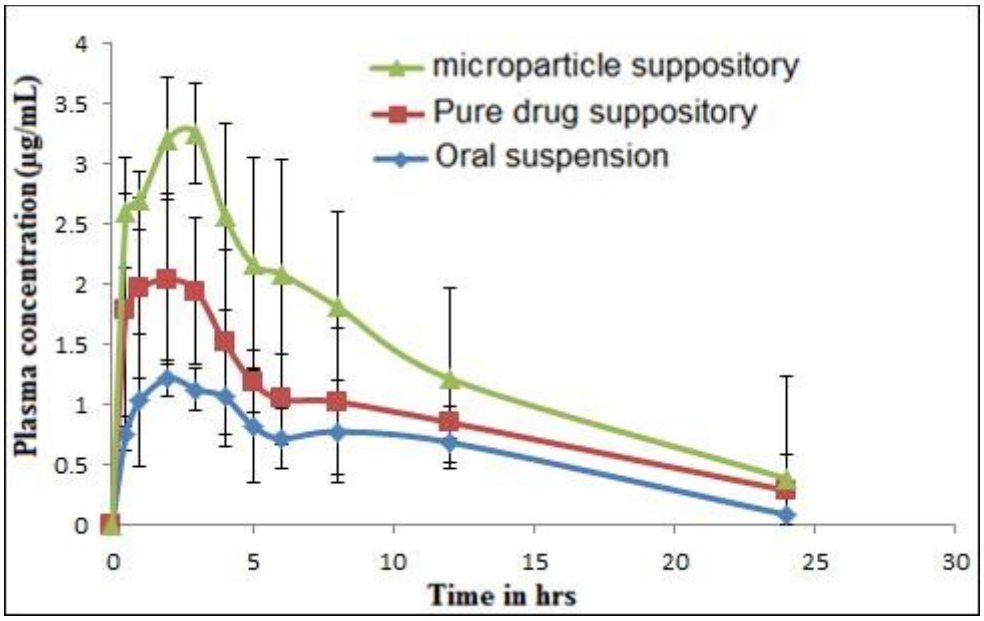

Fig. 3 Pharmacokinetic profile of felodipine formulations in rat

Increased plasma concentration after rectal administration of microencapsulated drug in the form of suppository could be attributed to avoidance of hepatic first pass effect, which was consequence of retaining the drug in the lower rectum i.e. by preventing the upward migration of the suppositories in the rectum by the aid of mucoadhesion. Higher absorption can also revealed the solubility-improving effect of microparticles.

Higher Cmax achieved after microparticle loaded suppository formulation administration could be result of escape of drug from hepatic metabolism. Due to mucoadhesive property of microparticles as well as suppository, drug was remained adhere to the site of application i.e. lower rectum lining. This was concern of holding the drug in the lower part of rectum and inhibited the ascending passage of the suppositories. Increased solubility of microparticles results into higher drug absorption from microparticle loaded suppository. Felodipine microparticle loaded poloxamer suppository would be useful to deliver felodipine in a pattern that allows better absorption.

4. Conclusion

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Despite complete absorption throughout the gastrointestinal tract, systemic availability of felodipine is drastically reduced by first pass metabolism. For improving bioavailability of drugs like felodipine, there is need of a formulation design considering use of rectal route and/or bioadhesive polymers to promote drug absorption. This approach based on rectal administration of felodipine alginate TKP microparticles loaded suppository can be promising alternative for improving systemic availability of drugs such as felodipine.

Conflict of Interest: None

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