RESEARCH ARTICLE

Development and *In vitro, In vivo* Evaluation of Fenugreek Mucilage based Intranasal Delivery of Venlafaxine Loaded Microspheres

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ABSTRACT

Objective: Gums are naturally occurring components in plants, which are essentially cheap and plentiful. They have diverse applications such as thickeners, emulsifiers, viscositifiers, sweeteners etc. in confectionary, and drug release modifiers in pharmaceutical dosage forms. Fenugreek gum microspheres of venlafaxine for intranasal delivery were prepared to avoid the first pass metabolism and increases bioavailability, as an alternative therapy to parenteral, and to improve therapeutic efficiency in treatment of depression, seizures.

Methods: Fenugreek mucilage was extracted from the fenugreek seeds. The microspheres were prepared using the extracted mucilage by spray-drying method. The microspheres were evaluated for characteristics like particle size, incorporation efficiency, swelling ability, zeta potential, *in-vitro* mucoadhesion, thermal analysis, XRD study and *in-vitro* drug release.

Results: Treatment of *in-vitro* data to different kinetic equations indicated diffusion-controlled drug delivery from fenugreek gum microspheres. The results of DSC and XRD studies revealed physical compatibility of venlafaxine with the fenugreek mucilage microspheres.

Conclusion: The microspheres had a good sphericity, a uniform distribution of particle size less than 10µm which is prepared by spray drying method. After getting contact with the nasal mucosa, microsphere formulations are believed to form viscous gel by withdrawing water from the nasal mucosa and interaction with cations present in nasal secretions.

Keywords: fenugreek mucilage, venlafaxine, microsphere, nasal microsphere.

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INTRODUCTION

Fenugreek (FG) (Trigonella foenum graecum Linn.) is an erect annual plant (height 30-60cm.) belonging to family Leguminosae (Fabaceae)[1,2]. Its green leaves and seeds are used in food and medicinal applications, which is an old practice of human history[3]. Naturally occurring polymers being biocompatible and biodegradable are currently extensively researched for the development of novel drug delivery system[4]. It was already investigated that fenugreek mucilage used as mucoadhesive aid in nasal drug delivery[5]. Fenugreek gum derived from the endosperm of the seed. It is a rich source of galactomannan which consists of the galactose and mannose monosaccharide unit (ratio 1.00/1.05). The primary structure of fenugreek gum is composed of α (1/4)-β-D-mannan backbone attached to single α-D-galactopyranosyl groups at the O-6 position of D-mannopyranosyl residues. Fenugreek has more water solubility due to high galactose content. It is reported that purified FG containing 0.8% protein[6]. Fenugreek seeds are rich in carbohydrate and especially mucilaginous fibre which is comprised of galactomannan[7]. Fenugreek mucilage reported as a gelling agent, mucoadhesive agent , binding agent [8,9].

In recent years nasal route received a great deal of attention and reliable method for systemic administration of drug. Nasal drug delivery has generated interest as an alternative route for administration of drugs and biomolecules that are susceptible to enzymatic degradation and first pass metabolism. Possible pathway for a drug to permeate across the nasal mucosa is passive transportation carriers mediated, transcytosis and transport through tight junctions. Nasal application of drugs is suggested to be the most viable alternative to the parenteral administration. As a site for drug delivery, nasal cavities have many advantages such as highly vascularized epithelial layer and wide absorption area. In addition, blood is drained directly from the nose into the systemic circulation, thereby avoiding first-pass metabolism in the liver and the intestine by enzymes and secretion by efflux transporter [10].

Venlafaxine is an antipsychotic drug. It is prescribed in schizophrenia, anxiety, depression etc. Venlafaxine is available as a conventional tablet in the market with multiple dose frequency. This drug shows a tendency of hepatic first pass metabolism. The taste of venlafaxine is bitter, so by encapsulating it into microspheres the taste of the drug may be masked[11]. Different delivery systems based on mucoadhesive polymers have been developed which are able to increase the residence time of the formulation at the absorption site of the drugs. The use of mucoadhesive system as microsphere provides a drug

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protection from enzymatic degradation and increases the contact time with the nasal mucosa. The aim of this study was to formulate the microspheres by spray drying technique based on the fenugreek mucilage and to evaluate the microspheres *in vitro, in vivo* in rabbits and thus improve bioavailability of venlafaxine by avoiding hepatic first pass effect.

MATERIALS AND METHODS

Materials

Venlafaxine obtained as a gift sample from Sandoz (Mumbai, India), fenugreek mucilage was extracted from seed obtained from local market. Ethanol (AR grade), Acetonitrile were of HPLC grade and purchased from S.D.Fine Chemicals Limited (Mumbai, India). All other materials used were of analytical grade and obtained from commercial suppliers.

Extraction of Fenugreek Mucilage

FSM was isolated from fenugreek seeds according to the previously reported literature[12]. Fenugreek seeds (200 g) were soaked in distilled water (1.5 l) at room temperature for 12 h and boiled using water bath until the preparation of slurry. After cooling, the slurry was cooled and kept in refrigerator overnight to settle out undissolved materials. The upper clear solution poured into thrice the volume of ethanol with continuous stirring. The precipitate was washed repeatedly with ethanol and dried at room temperature for 24h [5].

Drug – polymer Interaction

The physical mixture of drug and polymer were placed at 40°C±5 temp 75%RH humidity for 28 days and then interaction study was carried out by DSC

Differential Scanning Calorimetry (DSC)

Thermogram for venlafaxine, fenugreek mucilage and its physical mixture were obtained using DSC (Mettler DSC 1 star system, Mettler-Toledo, Switzerland). The drug was sealed in perforated aluminum pan and heated at constant rate of 10°C/min over the temperature ranges of 40-400°C(18). The measurements were performed under an inert atmosphere flushed with nitrogen at a rate of 20 mL/min.

Preparation of Microspheres

The formulations of microspheres as presented in Table 1 consisted of 1:1–1:5 drugs to polymer rations. Fenugreek gum was dispersed in water with continue stirring. Venlafaxine then mixed with the polymer solution. The solution of each batch was spray dried (LU222, Labultima, India) with the process parameters as follows: inlet temperature of 110°C, pump setting of 3-5 mL/min Spray pressure 2 kg/cm^2. The total volume of solution used for preparation of each batch was 400 mL[13,14].

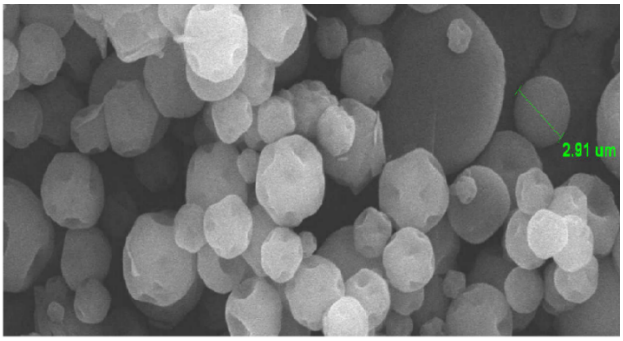


Figure 1: SEM image of spray dried microspheres

Table 1: Production yield of microspheres

Formulation code	Drug to polymer ration (%w/w)	Production yield* (% ± sd)
F1	1:1	26.23 ± 1.44
F2	1:2	11.23 ± 1.62
F3	1:3	14.57 ± 1.71
F4	1:4	25.66 ± 1.92
F5	1:5	18.68 ± 1.98

Characterization of Microspheres

Morphological Examination

The morphology of microspheres was examined by scanning electron microscopy. A small amount of powder was spread on an aluminium stub, which was placed after gold sputtering in SEM chamber (JSM 6390®USA). Photographs were taken at an acceleration voltage of 20 KV electron beam.

Percentage Yield, Drug Content and Incorporation Efficiency

The percentage of production yield was calculated as the weight of final product after drying, with respect to the total amount of dry starting materials. Venlafxine in microspheres of each formulation was extracted in phosphate buffer (pH 6.6). The concentration of venlafxine was determined using a UV spectrophotometer, at a wavelength 225nm (UV-spectrophotometer-1700, Shimadzu, Kyoto, Japan). Preliminary UV scan revealed that the presence of the polymer did not interfere with the absorbance of venlafaxine at 225nm.

The weighed number of microspheres were dissolved in distilled water and kept overnight to determine the drug content in each formulation. The resulting solution was filtered, and the filtrate was used to determine venlafaxine absorbance spectrophotometrically (UV 1700, Shimadzu, Japan) at 225 nm. Thus, the drug content was calculated.

The percent incorporation efficiency was calculated from actual drug content in weighed quantity of powder of microspheres (M_{actual}) and theoretical amount of drug in microspheres calculated from the quantity added in the fabrication process ($M_{therotical}$) as the following equation[13]:

$$\%\ Incorporation\ efficiency = \frac{Mactual}{Mtheoretical} \times 100 \quad ..(1)$$

Particle Size Measurement

A microscopic image analysis technique for determination of particle size was applied. The morphology and particle sizes were determined in a Motic DMW2-223 digital microscope (Motic Instruments Inc, Canada) equipped with a 1/399 CCD camera imaging accessory and computer-controlled image analysis software (Motic images 2000,1.3 version). The microspheres were dispersed on a microscope slide. A microscopical field was scanned by video camera. The images of the scanned field are analyzed by the software. In all measurements at least 100 particles were examined[14].

Zeta Potential Study

The microparticles were dispersed in ethanol. This dispersion was filled in zeta cell and placed in the Zeta

Sizer (Nano ZS, Malvern Instruments, UK). The potential was determined with the help of software.

***In-vitro* Mucoadhesion**

A freshly cut 2.00 cm^2 piece of sheep nasal mucosa was obtained and cleaned by washing with isotonic saline solution. One hundred milligram of microspheres were placed on mucosal surface which was fixed over poly ethylene plate for support. About 100 µL of simulated nasal electrolytes (SNES: aqueous solution containing 8.77 mg/mL NaCl, 2.98 mg/ml KCl and 0.59 mg/mL CaCl2 per liter) was put on microspheres and finally fixed at an angle of 45° relative to the horizontal plane. The nasal mucosa was thoroughly washed with phosphate buffer (pH 6.6) at the rate of 5 mL/min using a peristaltic pump. Sixty min after administration of microspheres, the concentration of drug in collected perfusate was spectrophotometrically determined. The microsphere amount corresponding to the drug amount in perfusate was determined. The adhered

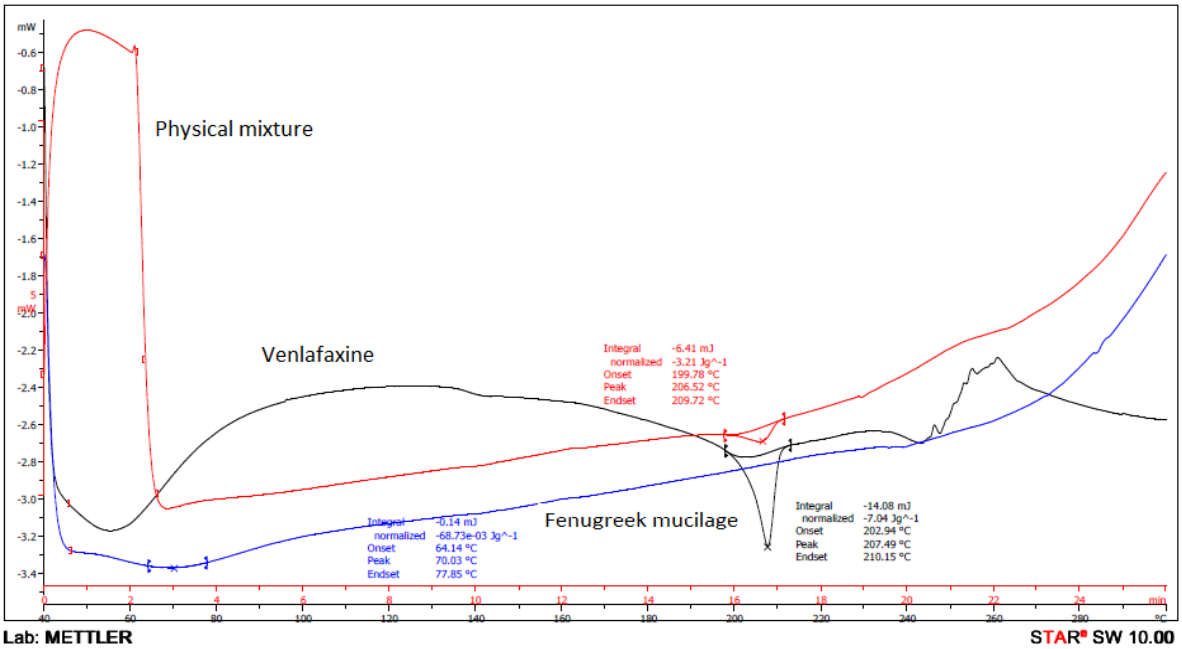


Figure 2: Differential Scanning Colorimetry study of the physical mixture, venlafaxine drug and fenugreek mucilage

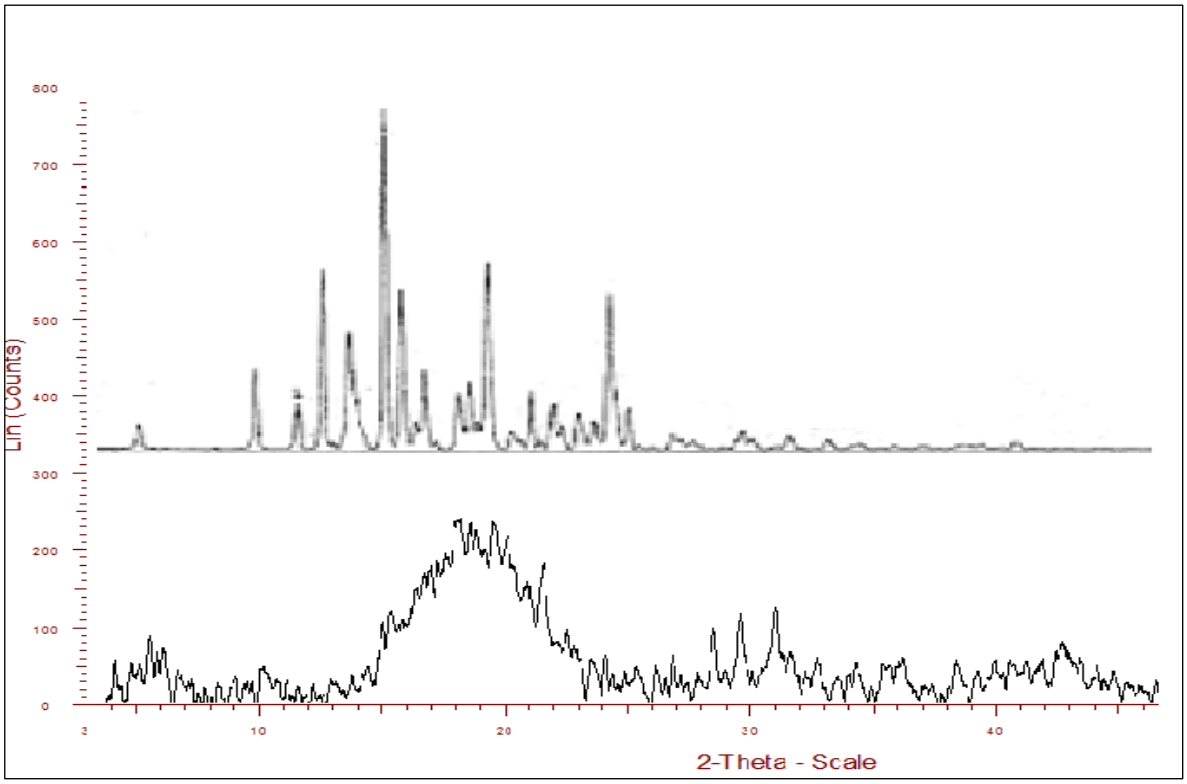


Figure 3: Overlay Xray Diffractogram of drug venlafaxine and microspheres

Table 2: Drug loading and entrapment efficiency of microspheres

Formulation Code	Drug Content (% ± SD)	Incorporation Efficiency (% ± SD)
F1	28.38 ± 0.59	43.84 ± 0.67
F2	24.68 ± 1.78	30.68 ± 1.13
F3	30.58 ± 2.03	22.87 ± 1.25
F4	62.06 ± 0.98	74.88 ± 1.23
F5	19.9 ± 1.82	50.89 ± 1.89

Table 3: Particle size, mucoadhesion and swelling index of microspheres

Formulation code	Mucoadhesion* (% ± sd)	Swelling index	Average particle size* (µm ± sd)
F1	78.80 ± 0.95	81.32	1.65 ± 1.43
F2	82.06 ± 1.33	85.51	6.75 ± 1.94
F3	88.34 ± 1.12	89.06	3.93 ± 2.23
F4	88.96 ± 1.34	94.93	5.61 ± 1.35
F5	89.98 ± 1.67	95.66	3.62 ± 2.67

microspheres amount was estimated as the difference between the applied microparticles amount and the flowed microparticles amount. The ratio of adhered microparticles was computed as percent mucoadhesion using following equation[15,16].

***In-vitro* Swelling Studies**

The swelling ability of the microspheres in physiological media was determined by swelling to its equilibrium. Accurately weighed amounts of microspheres (10.00 mg) were placed as Millipore filter NY 11, 0.22 µm using a Franz diffusion cell (10 mL) filled with phosphate buffer pH 6.6 and kept for 5 min. The following formula was used for calculation of degree of swelling:

$$\alpha = \frac{Ws - W0}{Ws} \quad .. (2)$$

where α = degree of swelling, Wo = initial weight of microspheres and

Ws = weight of microspheres after swelling[17].

X-Ray Diffraction (XRD) Studies

The X-Ray diffraction patter of pure venalfaxine and Venlafxine loaded microspheres, were evaluated by using an x-ray diffractometer. (Brucker Axs,08 Advance). Scanning was done up to 2θ of 5 and 25.

***In-vitro* Drug Release Study**

An *in-vitro* drug release test of the microspheres was performed using Franz diffusion cell with dialysis membrane (cut-off Mol. Weight.12000). The membrane was equilibrated before carefully dispersing the sample equivalent to 10 mg of drug onto the donor compartment. The donor compartment contained 3 ml of SNES and receiver compartment was filled with phosphate buffer solution pH 6.6 that was within the pH range in nasal cavity and maintained at 37°C±0.5°C. Samples were periodically withdrawn from the receptor compartment, replaced with the same amount of fresh buffer solution, and assayed by a spectrophotometer at 225.5 nm[18,19].

Histopathological Examination of Nasal Mucosa

The histopathological evaluation of tissue incubated in phosphate buffer (pH 6.6) for 6 h after collection was compared with tissue incubated in diffusion chamber with formulation. Tissue was fixed in 10% buffered formalin, routinely processed and embedded in paraffin. Sections were cut on glass slides and stained with haematoxylin and eosin. Sections were examined under a light microscope to detect any damage to the tissue[20, 21].

***In-vivo* Study**

The animal experiment was carried out in compliance with the protocol of Institutional animal ethical committee (17/CPCSEA under CPCSEA, India).

Subjects

Six New Zealand white strain rabbits with mean weight of 2.5 ± 0.3 kg were used. The rabbits were accommodated to the dosing for 1 month before the study to prevent withdrawal and defence reaction that may lead to inaccurate dosing. The rabbits were kept in single cage and fasted for 12 hrs before the study with free access of water during the experiments. A cannula was inserted into the marginal ear

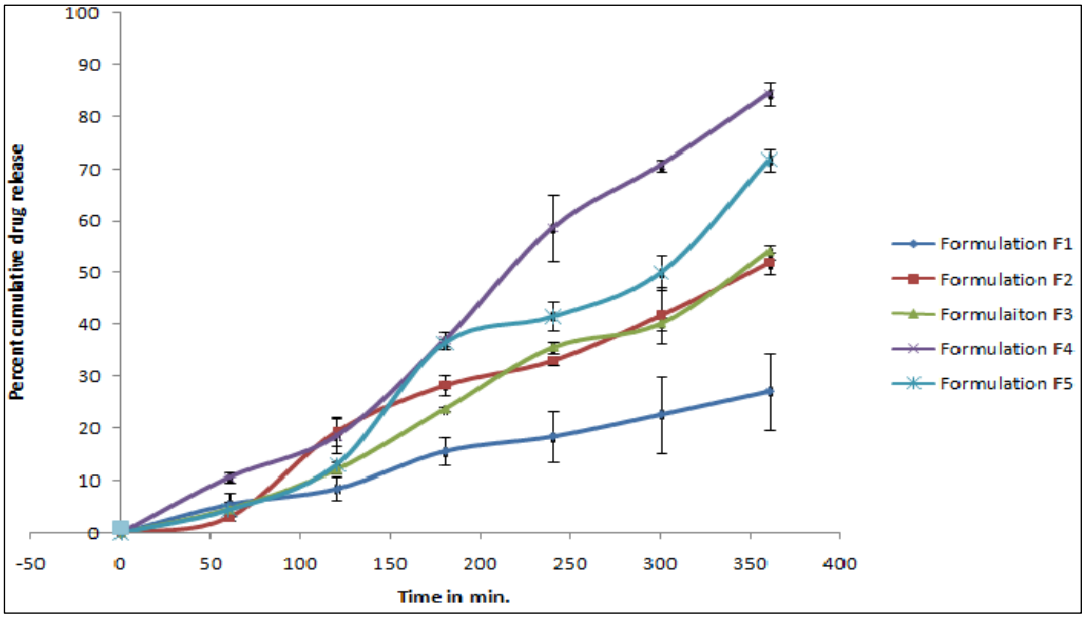


Figure 4: *In-vitro* drug release study of all formulations

vein for blood sampling and flushed with heparinised normal saline solution.

Study Design

In a crossover study with 1 week apart as a wash out period, the animals received 5 ml of oral drug solution by an oral tube and microspheres by nasal route of administration. Nasal microspheres were deposited into both the nostrils. Drug was administered to each group at the dose of 10 mg/kg. The blood samples (0.5 ml) were collected at the predetermined intervals for 360 min to analyze the concentration of venlafaxine in plasma[22,23].

Sample Collection and Analysis

After administration of the microspheres and the drug solutions by oral and intravenously, blood samples (0.5 ml) were collected at every 30 min time interval for 360 min from the marginal ear vein of the rabbits. Blood samples were mixed with dil. heparin solution as an anti-coagulant and then centrifuged at 5000 rpm for 25 min to obtain plasma samples. These samples were deep frozen at −20°C until HPLC analysis.

At the time of analysis 10.00 µl of 100.00 ng/ µl of methyl paraben as an internal standard (IS) was spiked in the 0.5 ml of thawed plasma. After the addition of IS the plasma was vortexed for 5 min. One millilitre of acetonitrile was added to the above spiked plasma. This sample was again vortexed for 5 min, centrifuged at 4000 rpm for 20 min, and filtered through a nylon membrane filter (0.45 µm); 20 µl of the resultant solution was analyzed by HPLC system using a Rheodine type manual injector. HPLC system (Agilent 1200 series) consisting of column C-18, Reverse phase column (Eclipse XDB, 5 µm, 4.6 mm × 150 mm, Singapore), Ezchrome Elite software, quaternary Pump, Model G1354A, and Ultra Violet variable wavelength Diode Array detector, Model G1315D. The detection wavelength was 226 nm. The mobile phase consisted of acetonitrile:phosphate buffer (10 mM, pH adjusted to 3 with orthophosphoric acid) 34:66 V/V (Xiao et al., 2008).Data treatment and statistical analysis[22,23].

Data Treatment and Statistical Analysis

Results from HPLC analysis were plotted as plasma drug concentration vs time. Non-compartment pharmacokinetic parameters including Tmax, Cmax, and AUC were estimated by Kinetica 5.0®computer software, which was a free trial version. The AUC values for each curve were calculated from zero to the last data point using the trapezoidal rule, with extrapolation to infinity. The $AUC_{0-\infty}$ values obtained from the curve were used to calculate the absolute bioavailability.

$$\%\text{Fabs} = \frac{\text{AUCOS/NP}}{\text{AUCIV}} \times 100 \quad ..(3)$$

Where % F_{abs} = absolute bioavailability; AUC_{OS} = area under the plasma concentration curve after oral administration; AUC_{NP}= area under the plasma concentration curve after nasal administration; and AUC_{IV}= area under the plasma concentration curve after intravenous administration.

RESULTS AND DISCUSSION

The spray-drying method described here appeared to be a suitable and simple technique to prepare fenugreek mucilage-based microspheres, loaded with venlafaxine. It is single step and rapid process, as it combines drying of the feed and embedding of the drug into a one-step operation.

Characterization of Microspheres

Morphological Examination

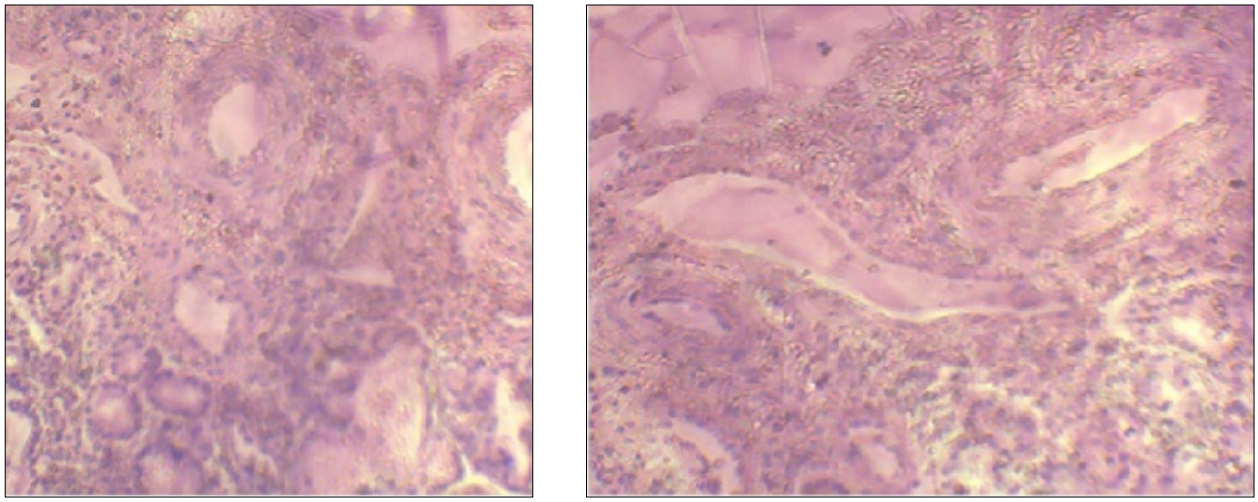
All formulations were oval to spherical in shape. They showed smooth surface (Figure 1), no rupture on the surface was seen such morphology may result in slow clearance and good deposition pattern in nasal cavity[24].(Figure 1)

DSC Study

DSC thermogram as shown in the image showed no extra peak or any dislocation of peak was not observed which confirms the compatibility of the excipients with that of drug. (Figure 2)

Percentage Yield, Percent Drug Content and Incorporation Efficiency

The production yield of spray-dried microspheres was between 11%-26% (Table 1). As previously observed, these low values can be justified by the low quantity of feed used for the preparation of each batch and by the structure of the



Untreated Mucossa Treated Mucossa

Figure 5: Histopathology images of untreated and formulation treated mucosa

spray drier, which lacked a trap to capture the smallest and lightest particles.

The determination of drug content showed uniformity in all formulations. It was in the range of 19-62% for all formulations. All microspheres had good incorporation efficiency between 22% and 75% (Table 2).

Particle Size Measurement

Particle size of microspheres is one of the most important characteristics as a nasal drug delivery. The mean particle size of microspheres ranged from 1.65 – 6.75µm. It has been suggested that 10 µm particles size is most suitable for nasal administration[13].

***In-vitro* Mucoadhesion**

Mucoadhesion studies was carried out to ensure the adhesion of the formulation to the mucosa for a prolonged period of time at the site of absorption. Percentage mucoadhesion was increased with increase in polymer concentration (Table 2). This could be because of more availability of hydroxyl functional groups for interaction with mucin. The functional groups available on the surface of the polymer favoring mucoadhesion are hydroxyl, carboxyl, amine and amide. Being carbohydrate, fenugreek mucilage is rich in functional group contents hence showed higher percentage of mucoadhesion[15].

***In-vitro* Swelling Studies**

In vitro swelling properties of the spray-dried microspheres were expressed as degree of swelling estimated. The maximum swelling (degree of swelling) was observed with microspheres containing highest concentration of fenugreek mucilage[17].

XRD Studies

The x-ray diffraction spectra recorded for drug and microspheres Figure 3. These studies are useful to investigate the crystallinity of drug in the microspheres. Venlafaxine has shown characteristic intense peaks between 2θ of 5 and 25, indicating amorphous nature of drug after entrapment into the fenugreek mucilage microspheres by spray drying.

***In-vitro* Drug Release Study**

The drug release profile from various formulations of microspheres is shown in Figure 4. Microspheres prepared with fenugreek gum moderately sustained the drug release to 6 h without any lag time. The rate and extent of venlafaxine release from microspheres significantly decreased with an increase in fenugreek mucilage concentration. In order to investigate the drug release mechanism, the release data were fitted to models representation zero order, first-order and Highuchi's square root of time.

The examination of coefficient of determination values indicated that drug release from the microspheres formulation followed the diffusion control mechanism (Highuchi model). A more stringent test was used to distinguish between the mechanisms of drug release. The release data was fitted to the Peppas exponential model,

$$Mt/M\infty = Ktn,$$

Where M_t/M_∞ is the fraction of drug released after time t; k is the kinetic constant; and n is the release exponent which characterizes the drug transport mechanism. The values for kinetic constant and release exponent are listed. The n values were in the range of 1.048–1.586 indicating that all the prepared formulations followed the non-fickion superclass-II diffusion controlled mechanism of drug release. On the basis of results of characterization of microspheres and *in vitro* drug release study F4 was considered as optimized formulation and found to give satisfactory results which make it suitable for nasal administration of venlafaxine[18,19].

Histopathological Evaluation

This study showed no sign of necrosis or irritation at the application site of the formulation. (Figure 5)

***In-vivo* Study**

Various pharmacokinetic parameters were investigated for formulation F1, in comparison with the oral route. showed higher plasma drug levels in nasal administration of drug through microsphere as compared to the oral administration of drug by solution. The Cmax values were 24.19±0.72 and 17.98±1.15 ng/ml for microsphere and for oral solution, respectively. The difference in Cmax values is significant at P\0.05. In addition, AUC0–360 values were 10805.31±30.82, 4278.96±25.25 and 5883.23±91.92 ng/ml/min for I. V., for oral and for microsphere mucoadhesive, respectively. These values corresponded to absolute bioavailability of 39.60 and 54.44% for oral and nasal microspheres, respectively, which are statistically significant ($P < 0.05$).

CONCLUSION

The results of our present study clearly indicated promising potentials of fenugreek mucilage-based microspheres for delivering drug intranasally and could be viewed as alternative to conventional dosage form. The microspheres had a good sphericity, a uniform distribution of particle size less than 10µm which is prepared by spray drying method. After getting contact with the nasal mucosa, microsphere formulations are believed to form viscous gel by withdrawing water from the nasal mucosa and interaction with cations present in nasal secretions. The resultant gel formation decreases the ciliary clearance rate and as a consequence the residence time of the formulation at the nasal mucosa is prolonged. The mucoadhesive properties of microspheres were attributed to spontaneous gel formation on nasal mucosa. Fenugreek gum is a biocompatible polymer; it does not cause any deleterious effect or toxic response in the nasal mucosal cavity even if used for prolonged periods as evaluated by histopathological studies. However, extensive pharmacokinetics and pharmacodynamic studies are required to establish a correlation, if any, before establishing venlafaxine nasal delivery as an alternative.

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